The influence of pollinators and herbivores on daisy flower colour community assembly patterns, plant species distributions and flower colour evolution in Namaqualand, South Africa

By

Jurene Ellen Kemp

Dissertation presented for the degree of Doctor of Philosophy in the Faculty of Science at Stellenbosch University.

Promotor: Prof. Allan G. Ellis

December 2018

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2018

Abstract

Plant species with flowers that are not phenotypically specialized are usually considered to interact randomly with the available pollinator species. Because such plants are considered generalist in their pollination interactions, they have received much less attention than those with complex specialised morphologies. However, plants with conserved generalist floral morphologies may still strongly filter the community of flower visitors through traits such as complex flower colour signals.

In my dissertation, I explored how various pollinators and herbivores interact with flower colour, and what the outcomes of these interactions are in terms of community assembly, plant species distributions, and floral trait evolution. Particularly, I focus on the diverse communities of daisies (Asteraceae) that annually flower en-masse in Namaqualand, South Africa. Because these daisies are highly reliant on pollination for persistence, we might expect selection for increased pollination efficiency and decreased floral herbivory.

In chapter 2, I assessed how flower colour patterns are assembled into communities. I found that daisy species within communities tend to share the same bulls-eye colour pattern, which suggests that evolutionary convergence, ecological filtering or facilitation is important in structuring communities. Further, I used bipartite interaction networks to confirm the role of pollinators in community assembly processes, and I showed that communities are dominated by a single pollinator species that interacted most strongly with the overrepresented flower colour pattern.

In chapter 3, I built on the findings from chapter 2, and asked whether pollinators have divergent colour preferences. I identified two dominant fly pollinator species that frequently

visited different dominant flower colours. Colour preference experiments showed that *Megapalpus capensis* had a strong preference for orange flowers, whereas *Corsomyza nigripes* had a strong preference for white flowers. Next, I quantified the densities of these two pollinator species independent from focal plant species in 100 communities, and I showed that pollinator densities are associated with the dominant flower colour in communities.

In chapter 4, I investigated the influence of herbivores on flower colour evolution. Many daisy capitula are only open for a few hours a day, and I modelled the reflectance spectra in multiple herbivore visual spaces of the petal surfaces that are exposed when daisies are closed. I showed that closing daisies tend to have cryptic lower petal surfaces, and I confirm my findings by conducting a series of experiments with herbivores.

In chapter 5, I assessed whether selection by various agents has resulted in the evolution of narrow pollination niche breadths. I calculated pollination niche breadths for all the daisy species in the networks from chapter 2, and showed that most daisy species interact with fewer pollinator species than expected under random visitation. Further, ecological specialization was associated with various visual signalling and reward-related traits. I conducted transplant experiments across a pollinator diversity gradient whilst altering plant competition, and showed that one *Gorteria diffusa* morphotype had a narrow fundamental niche that was not influenced by community context.

My findings challenge the prevailing perception of the generalised pollination systems of daisies, and I highlight the importance of flower colour in pollinator foraging choices and pollination niche breadth evolution.

Opsomming

Plantspesies met blomme wat nie fenotipies gespesialiseerd is nie, word dikwels aangeneem om lukrake interaksies met die beskikbare bestuiwers te hê. Omdat sulke plant as nie-spesialiste beskou word, het hulle aansienlik minder navorsingsaandag ontvang as plante met komplekse gespesialiseerde morfologieë. Ten spyte hiervan, kan plante met bewaarde nie-spesialis blommorfologieë steeds sterk die gemeenskap van blombesoekers filter deur ander eienskappe, soos komplekse blomkleurseine.

In my skipsie het ek ondersoek hoe verskeie bestuiwers en herbivore in interaksie tree met blomkleur, en wat die uitkomstes van hierdie interaksies is in terme van gemeeskapsvergadering, plantspesieverspreidings, en blomeienskap-evolusie. Ek fokus op die diverse madelief-gemeenskappe (Asteraceae) wat jaarliks in hul massas blom in Namakwaland, Suid-Afrika. Omdat hierdie plante uiters afhanklik is van bestuiwers vir hul voortbestaan, kan ons moontlik verwag dat seleksie ten gunste is van verhoogte bestuiwingseffektiwiteit en verlaagte blomskade.

In hoofstuk 2 het ek ondersoek hoe blomkleurpatrone in gemeenskappe vergader. Ek het gevind dat plantspesies binne hierdie gemeenskappe neig om dieselfde patroon te deel, en dit stel voor dat evolusionêre konvergensie, ekologiese filtering of fasilitering belangrik is in die strukturering van hierdie gemeenskappe. Verder het ek interaksienetwerke gebruik om die rol van bestuiwers in gemeenskapsvergaderingsprosesse te bevestig, en ek het gewys dat gemeenskappe gedomineer wod deur ‘n enkele bestuiwerspesie wat gereeldste die dominante kleurpatroon besoek.

In hoofstuk 3 het ek ondersoek of verskillende bestuiwers uiteenlopende kleurvoorkeure het. Ek het eers twee dominate bestuiwers geïdentifiseer. Kleurvoorkeureksperimente het aangedui dat *Megapalpus capensis* ‘n sterk voorkeur vir oranje blomme het, waarteenoor *Corsomyza nigripes* ‘n sterk voorkeur vir wit blomme het. Volgende het ek die digthede van hierdie twee bestuiwerspesies in 100 gemeenskappe gekwantifiseer, en ek het gewys dat hierdie digthede geassosieer is met watter blomkleur dominant is in ‘n gemeenskap.

In hoofstuk 4 het ek die invloed van herbivore op blomkleurevolusie ondersoek. Baie madeliefblomme is slegs vir ‘n paar ure daagliks oop, en ek het die refleksiespektra van die blomoppervlaktes wat sigbaar is wanneer blomme toe is, gemodelleer in die visuele sisteme van verskeie herbivore. Ek het gewys dat blomme wat snags toemaak neig om kriptiese onderste blomblaaroppervlaktes te hê, en ek het my bevindinge bevestig deur om eksperimente met herbivore te doen.

In hoofstuk 5 het ek ondersoek of seleksie deur die bogenoemde agente gelei het tot die evolusie van nou nisbreedtes. Ek het die bestuiwingsnisbreedtes van al die plantspesies in die netwerke van hoofstuk 2 bereken, en gewys dat nisse nouer is as wat verwag word onder lukrake bestuiwersbesoeke. Ek het ook gewys dat nisbreedtes geassosieer is met verskeie visuele seine en beloningsverwante eienskappe. Ek het oorplantingsekperimente gedoen oor ‘n bestuiwersdigtheidsgradiënt terwyl ek plantkompetisie verander het, en dit het gewys dat een *Gorteria diffusa* morfotipe ‘n fundamentele nou nisbreedte het wat nie deur gemeenskapskonteks beïnvloed word nie.

My bevindinge daag die algemene beskouing uit van ongespesialiseerde bestuiwingsisteme in madeliefspesies, en ek lig die belangrikheid van blomkleur in bestuiwersvoedingskeuses en bestuiwingsnisbreedte evolusie uit.

Acknowledgements

I would like to thank my supervisor, Allan Ellis, for patience, guidance and many interesting conversations. His office was always open, and he readily provided constructive feedback. He also allowed me the freedom to explore my own ideas, which allowed me to learn many new skills and to learn how to overcome a range of project obstacles.

I would like to thank my friends, particularly Nicolas, Suzette, Annique, Hannelize, Johanien and Melissa, for cheering me on over the last few years. I would also like to thank the various inhabitants of Room 1019, particularly Willem, Corneile and Christopher, for countless conversations on evolutionary theory and life.

I thank my parents and brother for their unfailing support and patience, and my amazing grandmother who unfortuntely could not witness the completion of this thesis.

The project was made possible by funding from the South African National Research Foundation (to JEK: 95181 & 111281; and to AGE) and Stellenbosch University (to AGE). Permits were provided by the Northern Cape conservation board, and SANParks graciously provided accommodation in Namaqualand. I would also like to thank Camille Madec and Bobo Ndlovu whom assisted with sampling.

This work was presented in part at the International Botanical Congress in Shenzen, China, in 2017.

Table of contents

List of tables

List of figures

Chapter 1

Introduction

Pollinators have long been recognized as important agents of selection on floral traits. More recently, the role of costly visitors like ineffective pollinators and herbivores, has also become apparent, and these act in concert with pollinators to determine the outcome of selection on floral traits. The majority of ecological and evolutionary pollination studies have centered around phenotypically specialized plant species (Ollerton *et al.* 2007), whereas phenotypically generalist species, that are assumed to have broad pollination niches, have received much less attention. However, selection can also act on reward or visual signalling traits, instead of on morphological traits, and this might be particularly relevant in taxa with conserved generalist floral morphologies.

In my dissertation, I explore how various mutualists and antagonists interact with flower visual signalling traits, and what the outcomes of these interactions are in terms of community assembly, geographic plant species distributions, floral trait evolution, and pollination niche breadth evolution. Particularly, I focus on daisies (Asteraceae) that annually flower en-masse in Namaqualand, South Africa.

In this introductory section, I briefly discuss what we know about the ways in which various biotic agents exert selection on floral traits, and how interactions with these agents can consequently result in the evolution of narrow pollination niche breadths and in community-level trait assembly patterns. I also include a section that explores the commonalities and differences between the visual systems of the pollinators and herbivores that are important in my thesis.

Selection by pollinators and herbivores on flower colour

The vast majority of angiosperms require biotic vectors to transport pollen between flowers (Ollerton *et al.* 2011). Because plants are dependent on pollinators for successful reproduction, pollinators can exert selection on floral shapes (Steiner and Whitehead 1990; Anderson and Johnson 2008; Cooley *et al.* 2008; Newman *et al.* 2015), rewards (Pauw 2006), and visual signal traits (Cooley *et al.* 2008; Campbell *et al.* 2012; Newman *et al.* 2012).

Flower colour often acts as a primary attraction signal to pollinators by indicating the availability or quality of reward (Odell *et al.* 1995; Hansen *et al.* 2012; Papiorek *et al.* 2013; du Plessis *et al.* 2018; Gray *et al.* 2018). Particular flower colours can influence pollinator behaviour in various ways. For instance, Hill *et al*. (1997) showed that individual honeybees sequentially visit either blue or yellow signals, and remain constant on one colour without sampling the other colour for reward. Moreover, colour patterns can also influence pollinator behaviour. For instance, concentric bulls-eye patterns can increase long distance attraction of pollinators (Koski and Ashman 2014), and small floral markings frequently act as nectar guides that help pollinators to orientate themselves when interacting with a flower (Johnson and Dafni 1998b; Dinkel and Lunau 2001; Leonard *et al.* 2013). Some markings, such as spots, can even elicit mating behaviour from deceived pollinators (de Jager *et al.* 2017), and this potentially increases both attraction rates and per-visit pollen transfer rates.

The variance between pollinator species in responses to flower colour partly results from pollinator groups differing in the type and number of colour receptors (see section below on visual systems). These differences can allow for private communication between flowers and particular pollinator groups; for instance, the tendency of bird pollinated flowers to be red or deep orange has been suggested to promote visual crypsis to bees, rather than to increase visitation rates by birds (Rodríguez-Gironés and Santamaría 2004; Shrestha *et al.* 2013;

Bergamo *et al.* 2016; Rivest *et al.* 2017). These visual system differences are also thought to partly drive 'floral syndromes' (Fenster *et al.* 2004), where floral traits converge across unrelated plant species when they are visited by the same functional group of pollinators.

However, the increased conspicuousness of flowers to pollinators often comes at the cost of attracting antagonists (McCall and Irwin 2006). Floral traits ultimately represent trade-offs between selection to attract pollinators and selection to avoid antagonists, and this trade-off is increasingly being recognised as an important determinant of floral trait evolution. Insect florivores and larger herbivores can directly reduce plant fitness by consuming pollen, ovules, and entire flowers (McCall and Irwin 2006). Florivory can also have indirect effects on plant fitness when floral damage alters pollinator regimes (Gómez 2003; Strauss and Whittall 2006; Sõber *et al.* 2010; De Waal *et al.* 2012). For example, de Waal *et al.* (2012) showed that mammalian herbivory led to the loss of apical flowers in *Babiana ringens*, which facilitated a pollinator shift through the evolution of a bird-perch that assists in sunbird pollination. Damage to flowers can also alter foraging decisions by pollinators, and consequently reduce visitation rates and seed set (Zangerl and Berenbaum 2009; Cardel and Koptur 2010).

Due to the costs associated with floral damage, we might expect plants to defend their flowers in various ways. Vegetative tissue is commonly chemically defended, and the same compounds involved in defending vegetative tissue are often present in floral tissue (Adler 2001; Strauss *et al.* 2004; Irwin and Adler 2006). Interestingly, Adler et al (2012) showed that *Nicotiana* plants that are reliant on pollinators have lower levels of defence compounds, which suggests a trade-off between chemical defence and pollination. A possible way to circumvent the trade-off between defensive compounds and pollinator attraction, particularly relevant in the context of flower colour evolution, is that plants can potentially avoid

herbivory through visual crypsis (Niu *et al.,* in press). In a similar way that plants can avoid less efficient pollinators by exploiting the variation in pollinator visual systems (Rodríguez-Gironés and Santamaría 2004; Shrestha *et al.* 2013; Bergamo *et al.* 2016; Rivest *et al.* 2017), plants can potentially also reduce the visibility of flowers to herbivores whilst attracting pollinators. Particularly, large mammalian herbivores are dichromats, whereas most pollinator species are tetra- or trichromats, which potentially allows for private communication between plants and pollinators. Additionally, many plants attract pollinators primarily through olfactory cues rather than visual cues (Johnson 1995), which can allow plants to reduce flower visual cues to avoid florivores.

The various processes that influence flower colour evolution (described above) often act simultaneously and the resultant flower colours are an optimization between mutualistic and antagonistic processes. The weight that each of these carries in the optimization likely varies with community context. For instance, if the abundances of florivores are high, we might expect floral traits that exclude florivores to evolve, potentially at the cost of visits by pollinators. Similarly, if pollinator abundances are low, we might expect highly attractive floral traits to develop which increases pollinator visitation rates at the cost of also attracting florivores. We might thus expect that spatial variation in pollinator and herbivore community composition should result in spatial variation in flower colour.

Evolution of ecological specialization

If pollinators and/or herbivores are selecting for particular flower colours, we might expect plant species to be ecologically specialized in their pollinator usage. Although the role of pollination specialization in plant speciation is well established (Grant and Grant 1965;

Stebbins 1970; Kay and Sargent 2009; Johnson 2010), the conditions that select for the evolution of narrow pollination niche breadths are not yet well understood (Fenster *et al.* 2004; Armbruster 2017). There are two broad pathways, that often act in concert, through which narrow niche breadths could evolve. That is, net fitness benefits can be increased either through an increase in fitness benefits or through a decrease in fitness costs. In the first pathway, selection acts on phenotypic traits that favour increased pollen transfer rates (i.e. increased fitness benefits) and, as by-product, plants become less adapted to other pollinator species. In the second pathway, selection acts on phenotypic traits that primarily reduce the number of visitor species to reduce the costs associated with having multiple visitor species (i.e. decreased fitness costs).

Adaptation to an effective pollinator to increase fitness benefits is often viewed as the primary pathway to pollination specialization (i.e. the most effective pollinator principle - Grant and Grant 1965; Stebbins 1970; Johnson 2010), but adapting to one pollinator group does not necessarily result in specialization. For instance, increased nectar production might increase visitation by an effective pollinator, but it might also increase the visitation rates of all other pollinator species. Thus, if selection favours an increase in fitness benefits (as opposed to reducing fitness costs), we only expect pollination specialization to occur if the adaptation to the primary pollinator reduces visitation rates by other pollinators as a by-product. For example, some orchids produce particular oils that increase visitation by oil-collecting bees, and consequently pollinators that do not collect oil do not visit those plant species (Pauw 2006). Here, selection thus primarily acts to increase visitation rates or morphological fit to pollinators, and not to reduce the breadth of the pollination niche.

Contrarily, in the second pathway, selection primarily acts to reduce the pollination niche breadth due to the costs associated with visits from multiple pollinators. For instance, if we

continue the earlier example where increased nectar volumes increases the visitation rates of all the pollinator species, we might expect the high visitation rates by many generalist pollinator species to result in increased heterospecific pollen transfer or pollen discounting. Consequently, these costs might select for a narrower fundamental pollination niche. We only expect adaptations that reduce the pollination niche breadth to evolve when the benefit of few pollinators outweigh the costs of losing pollinators (Aigner 2001). Particularly, we might expect narrow fundamental pollination niches to evolve when some visitors carry high costs, such as florivores, ovule feeders, or reward-robbers. Following from this, the general assumption that specialists are more efficient at utilizing their pollination resource than generalists (Armbruster 2017), does not necessarily hold true under the second pathway. Plants can reduce their pollination niche without improving the efficiency of the remaining pollinator(s). This is likely to occur when visitation from the primary pollinator species results in maximum seed set, and visits from additional species mainly incur costs.

Further, we only expect plants to specialize on reliable pollinator species; that is, those that emerge annually and that occur in sufficient abundances. However, reliant and abundant pollinators are usually utilized by many plant species in a community, and often the most specialist plant species are visited by the most generalist pollinator species (Bascompte *et al.* 2003). This should result in high levels of heterospecific pollen transfer (Arceo-Gómez *et al.* 2016), unless traits evolve that result in plant species placing their pollen on different parts of the pollinator's body (Pauw 2006; Muchhala and Potts 2007), or traits evolve that result in pollinator constancy (Gegear and Laverty 2005). Thus, narrow fundamental pollination niches can be costly, particularly if pollinators are unreliable in their occurrence or if pollinators visit many other plant species, and thus narrow niches should only evolve when being generalist is even more costly (Aigner 2001).

The evolution of pollination niche breadths is thus highly reliant on the surrounding community context. If competition between plant species for pollinators is prevalent, we might expect traits that increase fitness benefits to evolve. If many pollinator species are available and pollinator visitation rates are high, we might expect traits to evolve that exclude visitor species. If pollinator visitation rates are low and plants are pollen limited, then we might expect broad fundamental niches to evolve.

Community assembly patterns and processes

If pollinator species distributions show geographic structure and if pollinator species have divergent colour preferences, we might expect communities to be non-randomly assembled across a region. The influence of this on plant trait community assembly patterns has become increasingly clear (e.g. McEwen and Vamosi 2010; De Jager *et al.* 2011; Muchhala *et al.* 2014; Runquist *et al.* 2016). Moreover, pollinators can influence the assembly of flower colours across communities through either ecological processes, such as filtering, facilitation or competitive exclusion, or through evolutionary processes, such as trait convergence or character displacement (Sargent and Ackerly 2008).

If communities are dominated by a single pollinator species, or a single functional group, that has strong flower colour preferences, we might expect plant species within communities to share the same flower colour. In contrast, if many different pollinator species are available, and they have divergent colour preferences, then we might expect communities to consist of many different flower colours. However, multiple processes can lead to the same emergent community-level trait assembly patterns (Sargent and Ackerly 2008), and the inferred processes are rarely verified through experimental work. For instance, if many plant species

within a community share the same flower colour, this could result from evolutionary convergence, ecological filtering or facilitation. I discuss these processes and their emergent community-level patterns fully in Chapter 2 (see Figure 2.1).

Despite multiple community assembly processes resulting in similar trait assembly patterns, this approach is a useful first step in assessing whether a particular selective group, such as pollinators, are important in community-level processes. It can also show what traits are subject to selection or ecological sorting. Because of this, I begin my thesis by assessing community flower colour assembly patterns and processes, and I subsequently build my thesis around these findings.

Visual systems of relevant taxa

Pollinators: Bees

Many studies have investigated the bee visual system, particularly in honeybees and bumblebees. Bees have trichromatic vision (Daumer 1956, Von Frisch 1965) with ultraviolet, blue and green receptors. Bees use a chromatic opponency system to process colour, i.e. the receptors act in an antagonistic manner, and they have an achromatic pathway that acts through the green receptor. Both chromatic and achromatic pathways are involved in the processing of colour stimuli (Giurfa *et al.* 1996, 1997). Chittka (1992) proposed to assess bee colour vision using a hexagon visual space model (Fig. 1.1), and this has become the generally accepted way to model bee vision, particularly as the distances in the hexagon colour space have been validated with experimental work.

Honeybees have been shown to exhibit innate colour preferences. Menzel's (1967) experiments showed that honeybees learn to associate food with colour faster for some wavelengths than for others, and these colours match the preferences of naive bees (Giurfa *et al.* 1995). Further, honeybees exhibit improved colour discrimination after conditioning (Avarguès-Weber and Giurfa 2014), which suggests that neural processing might be important in their perception of colour. Experimental work has also shown that bee species vary in their ability to distinguish between different colours (Garcia *et al.* 2017), and thus experimental work is always required to verify exact discrimination abilities of any particular species.

Pollinators: Flies

Diptera have unique visual attributes compared to other taxa, and their visual processing is understudied and potentially complex. As with other insects, flies have compound eyes, but in contrast to most other insects, flies have an open rhabdomen and thus have neural superposition eyes, i.e. signals captured by different eyes are neurally pooled. Flies possess eight rhabdomere types, i.e. photoreceptors, of which the first six receptors (R1-6) have been shown to be important in motion detection, whereas the other two are important for colour detection (R7-8). As the two colour receptors each have two variants, this results in four colour receptor classes (Troje 1993). Additionally, many fly species have corneal colour filters; for instance, the eyes of Tabanidae flies often reflect green wavelengths, resulting in the eyes having a green appearance (Lunau & Knuttel 1995). Interestingly, some fly species have multiple different corneal filters within an eye, such as *Poecilobothrus nobilitatus* that has red and green striped eyes (Lunau & Knuttel 1995, Lunau 2014). However, the function of these multi-coloured eyes has not been investigated, and it is not understood how this influences colour perception.

Colour perception in flies is thought to be tetravariant (Fig. 1.1); that is, they perceive flower colour in one of four colour categories, rather than perceiving colour gradients. According to Troje's (1993) experiments, *Lucilia* flies could not distinguish between colours of wavelengths that were very different from one another, and the subsequent tetravariant colour model assumes an opponency mechanism within each receptor class. Although these results have been confirmed by Fukushi (1994) for *Lucilia*, tetravariant colour perception has not been tested for any other fly species.

Despite the lack of research on colour perception mechanisms in flies, many studies have shown that flies exhibit colour preferences (see Lunau 2014 for full discussion). Thus,

although all evidence shows that flies have the ability to perceive colour and evidence shows that they exhibit preferences in the wild, we do not fully understand how well they can discriminate between colours, under what conditions they can distinguish colours from the background, and to what extent colour plays a role in their foraging decisions.

Herbivores: Ungulates

Ungulates are dichromats with refractive cornea eyes that have short and medium/long wavelength receptors. The receptor peak absorbances have been identified for sheep, goats, and horses (amongst others), and the maximum sensitivities of the receptors vary between species (Fig. 1.1). The long wavelength receptor is used for achromatic vision, and both are used for chromatic vision. If both cone types are stimulated equally by a particular wavelength of light, then dichromats cannot distinguish this signal from achromatic light (Jacobs 1981), and this neutral point is only found in dichromatic animals.

Horses have commonly been used to test ungulate vision. They have been shown to be able to discriminate between various colours and grey (Carroll *et al.* 2001; Geisbauer *et al.* 2004; Roth *et al.* 2008), which shows that they have colour vision. However, they do not discriminate well between different shades of grey, indicating that they have high receptor noise levels (Geisbauer *et al.* 2004). Further, receptor peak absorbance values have been quantified for various ungulate livestock (Jacobs *et al.* 1998), but these behavioural tests have rarely been done for wild animals.

Biochemistry of flower colour

Flower colour pigments can either be flavonoids, carotenoids or betalains. Flavonoids, including anthocyanin, can produce yellow, pink red and blue colours, whereas carotenoids

produce yellow and red. Betalains are only found in Caryophyllales and never co-occur with flavonoids.

The loss of anthocyanin and/or carotenoids result in yellow or white flowers (Barb *et al.* 2008), and anthocyanin pigment gains or losses can be induced by a change to single transcription factors. Transitions between flower colours can occur when anthocyanin pigments are not produced anymore due to changes in the functionality of genes or due to changes in the regulation pathways. Other colour shifts, such as from blue to red, can occur when a change in the anthocyanin class occurs; that is, an increase in the number of hydroxyl groups will shift the colour towards shorter wavelength colours (see Wessinger & Rausher 2012 for discussion). Smith & Rausher (2011) showed that the shift from blue to red anthocyanins in *Iochroma* resulted from multiple genetic changes, whereas Hoballah *et al.* (2007) showed that the shift to white flowers in *Petunia* resulted from a single gene inactivation. The reversion of pigments to a previous colour is not seen very commonly, even when the loss of colour resulted from a mutation to a single gene, and this is thought to result from the accumulation of mutations in the network of genes that are involved in pigment production (Zufall & Rausher 2004). However, work on the Solanaceae has shown that plant species can develop the same colour using different pathways; that is, some plant species produce red flowers by producing red anthocyanins whereas others produce red flowers by producing purple or blue anthocyanins along with orange carotenoids (Ng & Smith 2016).

Further, the colour that anthocyanins reflect are influenced by the pH in the vacuole where the pigments are stored; that is, low pH levels can shift colours toward long-wavelength colours and high pH levels can shift colours towards short-wavelength colours (Zhao & Tao 2015). In contrast, carotenoids are stored in chromophores and the pH levels in vacuoles thus do not influence their reflectance spectra.

There are thus various ways in which plant species can transition between flower colours, of which some transitions are more likely than others.

Study system: Namaqualand, South Africa

Climate and Geology

Namaqualand is a biogeographical subsection of the Succulent Karoo biome of South Africa that spans approximately 45,000 km^2. The region is bound in the north by the Gariep River, in the west by the Atlantic ocean, in the south by the Olifants river and Bokkeveld escarpment, and is loosely bound in the east by the Bushmanland plains (Desmet 2007).

My thesis work was primarily conducted in three of the seven Namaqualand bioregions; that is, the Sandveld along the coast, the Kamiesberg towards the interior, and the Hardeveld that lies in between. The sands on the coastal plains on the west are mainly from marine origin, and range from young, white, calcareous sands (<100,000 y.o.) to older, red, acidic sands (ca. 2 M.y.o.) (Desmet 1997). In the mountainous Kamiesberg, soils are granite gneiss derived, and can be sandy to loamy. The Kamiesberg is characterised by intrusions of igneous rock, particularly granite outcrops. Soil depths range from only a few centimeters to several meters deep.

The winter rainfall of this arid region was initiated around the start of the Miocene due to the Antarctic glaciation (Zachos *et al.* 2001), changes in sea surface temperatures (Siesser 1980, Zachos *et al.* 2001), and the character of the high-pressure cells over the southern oceans (Siesser 1980, Linder 2003). Contemporary rainfall in Namaqualand comes from cold fronts from the southern oceans that results in light, widespread showers during winter. The mountains receive more rain than the plains, and the annual rainfall is highly predictable albeit low (i.e. 100-250 mm between May and September). The cold Atlantic ocean moderates the temperatures of Namaqualand, keeping average summer temperatures below

30°C (Desmet 2007), and often produces a fog bank on the coastal plains that moistens the soil.

Flora

The Succulent Karoo flora contains more than 6,000 species from 168 families (Driver *et al.* 2003), and Namaqualand contains ca. 3500 species from 135 families, of which a quarter of the species are endemic (Desmet 2007). Lineages that are endemic to the Succulent Karoo are mostly less than 10 My old, and none are older than 17.5 My, making it a relatively young biome that originated in the late Miocene during the aridification of this region (Verboom *et al.* 2009). In Namaqualand, the Asteraceae is the most species-rich family, followed by the Aizoacae, Iridaceae, Schrophulariaceae, and Fabaceae (Born *et al.* 2007). Despite the exceptional diversity of daisies in this region, we have little understanding of what has promoted their diversification. Although more than half of the flora in this region is small leaf-succulents or geophytes (Desmet 2007), this region is know for its austral spring mass-flowering displays that are dominated by annual Asteraceae plants (Fig. 1.2). Particularly, these floral displays are dominated by actinomorphic flowers that offer pollen and/or nectar as reward (Struck 1994, Fig. 1.3).

Actinomorphic flowers are often assumed to be associated with broad fundamental pollination niches (Fenster *et al.* 2004); that is, any insect can access nectar and pollen rewards and thus potentially act as pollinator. Because Namaqualand daisies are highly reliant on pollination for persistence, we might expect selection for increased pollen transfer rates, which could potentially result in pollination specialization. Daisies present an interesting scenario where all insects that land on an inflorescence will make contact with the reproductive structures, and the general layout of the inflorescence does not seem to be genetically labile. However, selection can act on other attraction or repellant traits, such as

nectar chemistry, flower colour, and nectar accessibility. Struck (1994) suggested that the longer nectar tubes of disc florets of *Senecio*, *Pteronia* and *Othonna* Namaqualand daisy genera act as filters to prevent short-proboscid insects from accessing nectar. Further, Ellis and Johnson (2009) showed that *Gorteria diffusa* has 14 different geographically structured floral morphotypes, which is suggestive of local adaptation to spatially varying pollinator communities. This thus suggests that pollinators might be exerting selection on floral traits, other than floral symmetry, in Namaqualand daisies. However, the extent to which this applies across Namaqualand taxa is not known.

Flower-visiting insects

Although naturalists and researchers have collected extensive information on plants in the Greater Cape Floristic Region for nearly 350 years, very little is known about the insects that interact with these plants. Struck (1994) conducted some of the earliest work on the pollination ecology of plants that flower during the Namaqualand mass display, and found that pollinator abundances were generally low. Further, he showed small scale variation in pollinator community composition, which he attributed to the fine-scale spatial variation in suitable nesting sites or nesting substrates (i.e. abiotic requirements) (Struck 1994). His study on Asteraceae and Aizoaceae revealed that more than 300 insect species from 41 families visited his focal plant species. He found that Bombyliidae flies were as abundant as bees, and they were more likely to be active during the frequent adverse weather conditions than bees. Beetles, particularly Hopliini (Scarabidae), also frequently visited Asteraceae and Aizoaceae flowers, but as they usually ate pollen, ovules or petals, they are unlikely to be efficient pollinators (Struck 1994). Further, butterflies were mostly absent from the region (Struck 1994).

Subsequent work in Namaqualand has shown the importance of bee flies (Bombyllidae) in the pollination and evolution of Namaqualand daisies (Fig. 1.4). Particularly, work has focused on *Megapalpus capensis*, a bee fly that visits various floral morphotypes of *Gorteria diffusa* (Asteraceae). This bee fly has been shown to exhibit mating behaviour on floral ornaments in *G. diffusa,* but also frequently visits non-deceptive floral forms of various daisy species (Ellis and Johnson 2009; De Jager and Ellis 2013). The genders of *Megapalpus capensis* also show different preferences for different floral traits (De Jager and Ellis 2012), and the flies do not exhibit floral constancy (Ellis and Johnson 2012). Despite this, *M. capensis* seems to be the dominant pollinator of multiple orange-flowered daisy species (Ellis and Johnson 2009; De Jager and Ellis 2014). Other than the work on *M. capensis* behaviour and how this influences floral trait evolution in *G. diffusa*, nothing is known about which pollinators are dominant in this region, how they are spatially distributed, how they influence community trait assembly patterns, or what their biotic and abiotic requirements are.

Objectives of research chapters

The overarching aim of this thesis is to assess the influence of pollinators and herbivores on flower colour community assembly patterns and flower colour evolution (Fig. 1.5). First, I assess how flower colours are assembled into communities. If pollinators (or herbivores) are influencing selection or ecological sorting of flower colour, then I expect non-random assembly of this trait across communities. Next, I use various modelling and experimental approaches to assess how pollinators and herbivores interact with (and potentially exert selection on) different flower colours. Finally, I assess whether these interactions have led to the evolution of narrow fundamental pollination niches.

In chapter 2, I assess whether biotic interactions influence community flower colour pattern assembly processes. Here, I first determine whether the regional pool of daisy species comprises distinct floral phenotype groups (based on colour bulls-eye patterns), and I assess whether flower colour pattern is phylogenetically conserved or not. Next, I investigate how these groups are assembled into communities using null models. This is done based on raw reflectance spectra, and through the visual systems of bees and flies. I predict that if community assembly patterns result from competitive exclusion, or if plant species exhibit character displacement, the regional plant species pool will be evenly distributed into flower colour pattern groups and colour pattern groups will be overdispersed in communities. If facilitation, filtering or convergence is driving community assembly patterns, then I expect the majority of plant species to share colour patterns and I expect clustering of colour pattern groups within communities. To verify that the community assembly processes result from interactions with pollinators, I assemble multiple pollinator visitation networks and assess whether network structure conforms to expectations given processes identified by the community assembly models

In chapter 3, I build on the pattern-based results from chapter 2, and I experimentally test whether different pollinator species have different colour preferences. I ask whether geographic structure in pollinator occurrences, along with divergent colour preferences, matches the geographic structure of flower colour. To do this, I focus on two prominent community types in Namaqualand identified in chapter 2, those dominated by orange flowers and those dominated by white, and I focus on pairs of closely related plant species within two different plant genera. I show that the bee fly *Megapalpus capensis* is the dominant visitor to orange flowers and *Corsomyza nigripes* is the dominant visitor to white flowers. I present individuals of these two fly species with a choice between orange and white flower pairs from the two plant genera in caged experimental arenas. I further test whether choices are different when background contrast (i.e. flower detectability) changes, and if pollinators are able to learn to use a different flower colour. Next, I quantify pollinator species abundances independent from the focal plant species, and I then test whether pollinator species densities predict the dominant flower colour in communities.

In chapter 4, I assess the influence of herbivores on flower colour evolution in daisies. Many daisies are only open for a few hours every day (usually between 10 am and 4 pm), and I test whether the lower petal surfaces of closing daisies are cryptically coloured. As these daisies are annuals, the loss of the entire reproductive structure might lead to strong selection to avoid herbivory. Closing is conserved at the genus level in daisies, and lower petal surface colouration should not be under selection from pollinators. I collect reflectance spectra of upper and lower petal surfaces for multiple daisy species and we then model these spectra in various herbivore vision models. If lower petal surfaces in closing daisies are adapted to be cryptic, I expect these surfaces to be less visible in herbivore vision than those of non-closing daisies. Further, if lower petal surfaces of closing daisies are adapted to be cryptic, their lower petal surfaces might produce colour pigments different to those of the upper petal

surfaces, and I do not expect this in non-closing daisies. I verify my modelling results by performing choice experiments using vertebrate herbivores. If daisy inflorescences are targeted by herbivores, which should act as selective pressure, I expect them to be eaten more frequently than daisy leaves. If closing daisies have cryptic abaxial petal surfaces, I expect daisy flowers to be eaten at the same rate as leaves when they are closed.

In chapter 5, I investigate the fundamental pollination niche breadths of Namaqualand daisies. If pollinators and herbivores are exerting selection on flower colour and other floral traits, we might expect narrow evolved fundamental niches. I use the bipartite interaction networks from chapter 2 to calculate ecological specialization and I test whether observed niche breadths result from random interaction with pollinators. Next, I test whether Namaqualand daisies represent global outliers in terms of ecological specialization by comparing their niche breadths to actinomorphic and zygomorphic species from other regions. I then use two approaches to test whether narrow observed niche breadths represent fundamental or realized niches. Firstly, I test the expectation that if niche breadths are evolved and not influenced by ecological context, niche breadths should be associated with various floral traits. Secondly, I use two morphotypes of *Gorteria diffusa* in transplant experiments across a pollinator diversity gradient whilst altering heterospecific plant competition intensity. If observed niche breadths in these morphotypes are evolved, I expect these plants to use the same pollinator species across treatments.

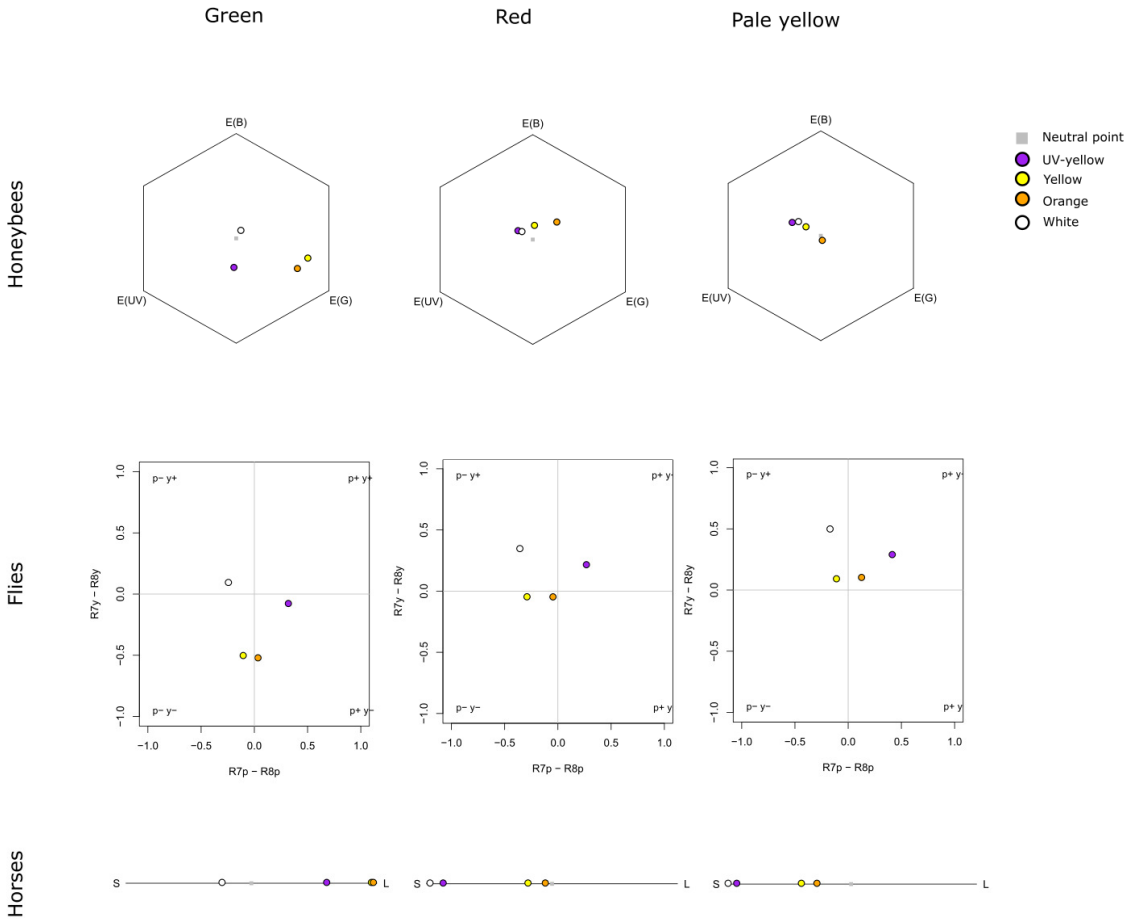

Figure 1.1. Reflectance spectra typical of Namaqualand daisies are shown in different visual systems. I used real reflectance spectra from UV-yellow, yellow, orange and white flowers, and I modelled them in bee, fly and horse visual space using three hypothetical background types (i.e. green leaf, red, pale yellow). Daisies occur on various soil types, and the contrast between flowers and background potentially shows spatial variation. In bee and horse visual space, dots closer to the centre of the hexagon (bee space) and line (horse space) are less distinguishable from the background. Flies are tetravariant, and colours within a quarter are not distinguishable from one another.

Figure 1.2. Annually, hundreds of daisy species flower simultaneously in high densities in Namaqualand, South Africa.

Figure 1.3. The daisy inflorescence layout is shown. Daisies have composite flowers that usually consist of ray florets with large fused petals that occur on the outer edge of the capitulum, and disk florets in the center of the capitulum with small petals fused into a nectar tube. Some daisy species consist only of disk florets. Anthers are contained in the nectar tube and releases pollen onto the stigma as the stigma grows. When pollen is removed from the outside of the stigma, the stigma opens up and becomes fertile. Daisies are functionally actinomorphic and any insect that lands on the flowerhead can act as pollinator. The photo shows a *Gorteria diffusa* (soeb morphotype) inflorescence that is visited by a *Megapalpus capensis* bee fly in an experimental setup.

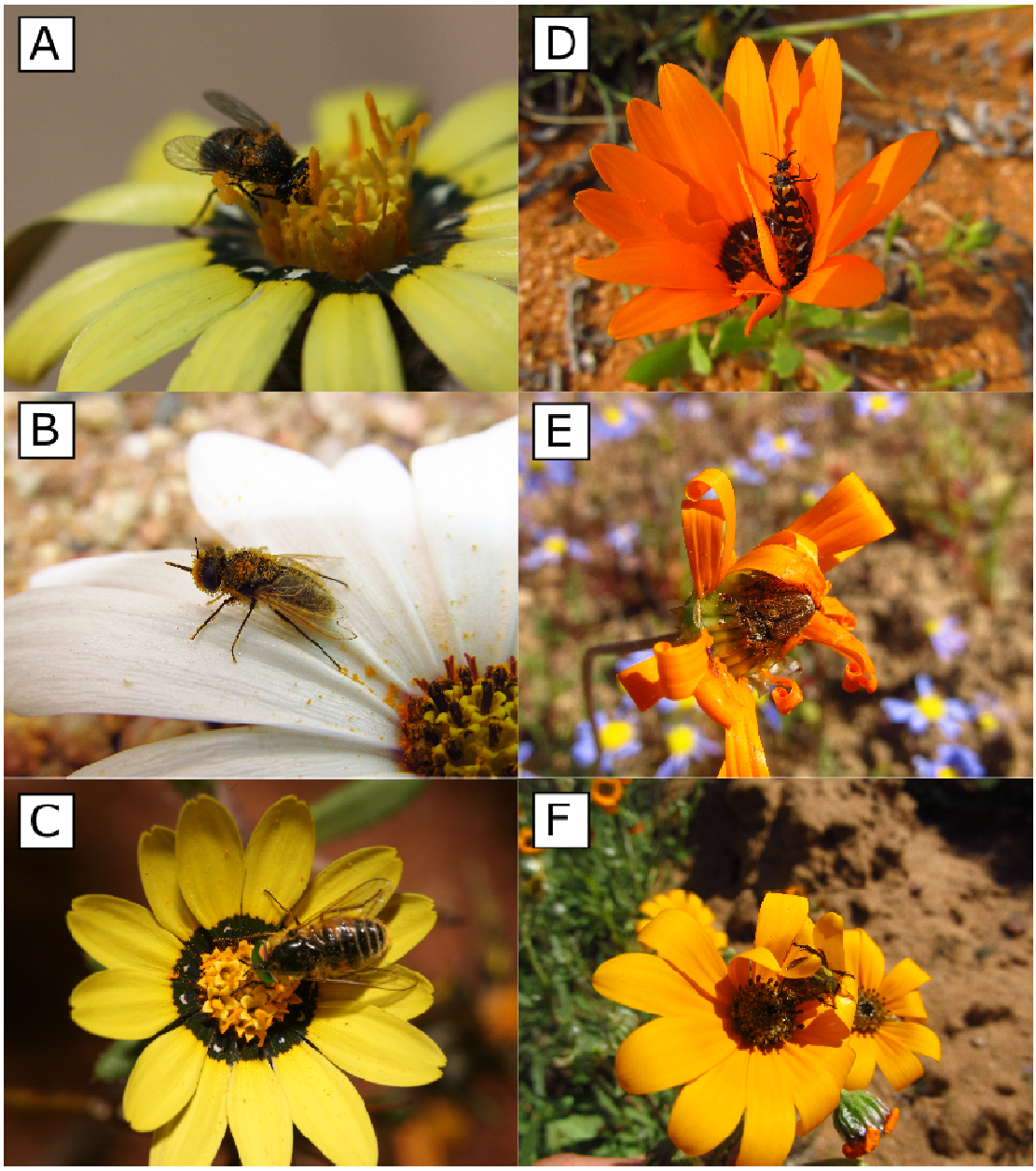

Figure 1.4. Various insects that visit Namaqualand daisies are shown. (A) *Megapalpus capensis* (Bombyliidae) is feeding on *Gorteria diffusa* (soeb morphotype) – note how its head and upper abdomen makes contact with the anthers when it is trying to access the low nectar volumes. (B) *Corsomyza nigripes* (Bombyliidae) is covered in pollen. (C) *Rhigioglossa* sp. (Tabanidae) occur in high abundances on the coastal plains, and are often seen covered in pollen (this photo was taken by Bruce Anderson). (D) Meloid beeltle feeding on petals. (E) A

monkeybeetle (Scarabidae) is embedded in the daisy capitulum whilst feeding on ovules with only the back of its abdomen visible. (F) A monkeybeetle (Scarabidae) is damaging ray florets.

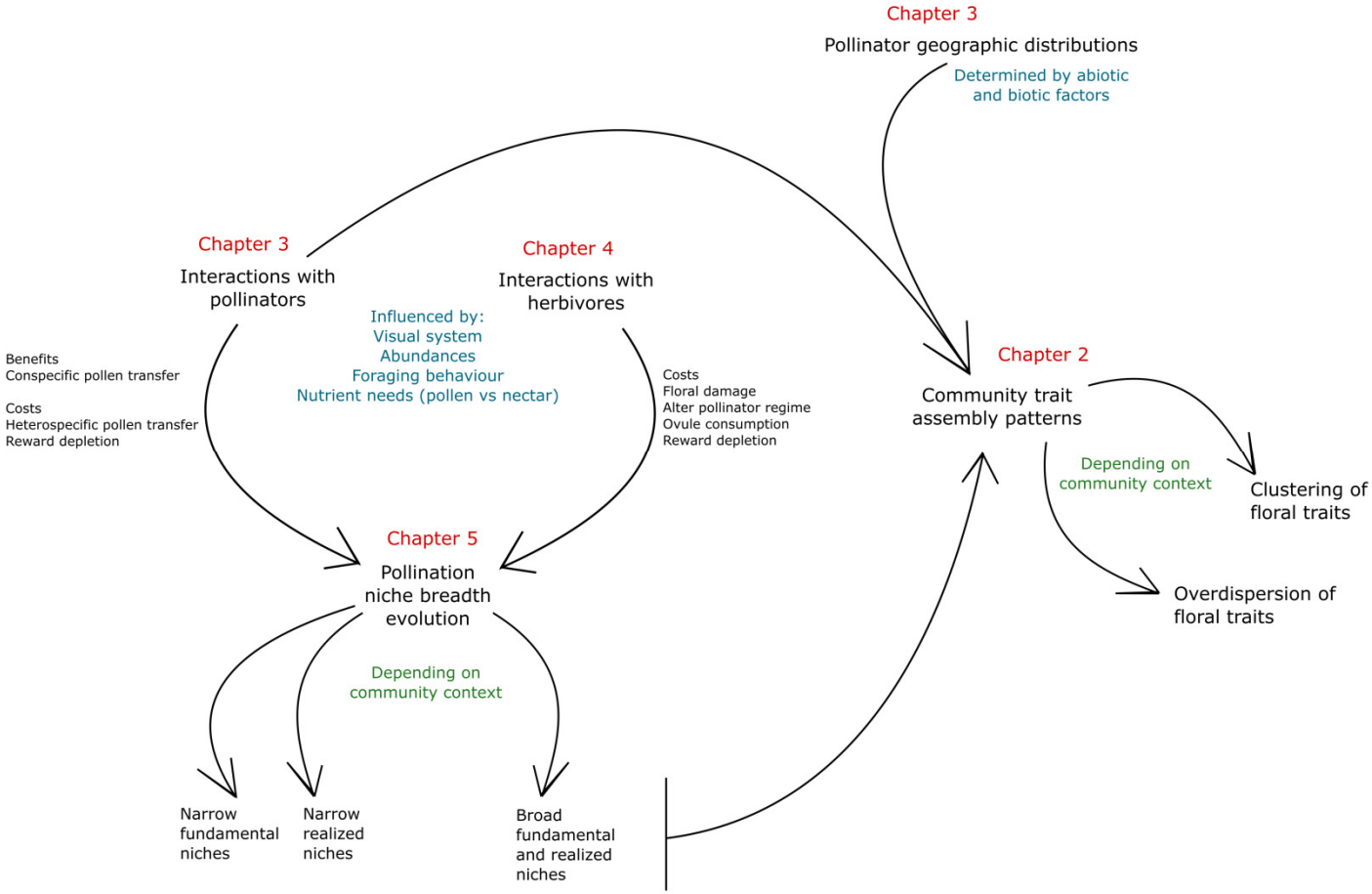

Figure 1.5. A map of the pathways connecting the different thesis chapters is shown. Pollinators and herbivores exert selection on flower colour, and the outcome of selection is highly dependent on community context. This results in niche breadth evolution, and along with geographic species distributions, these interactions also influence community assembly processes.

Chapter 2

Dominant pollinators drive non-random community assembly and shared flower colour patterns in daisy communities.

Jurene E Kemp, Nicola G. Bergh, Muri Soares & Allan G. Ellis

This paper has been accepted at Annals of Botany (doi: 10.1093/aob/mcy126)

Abstract

As most plants rely on pollination for persistence in communities, pollination interactions should be important determinants of plant community assembly. Here, trait and phylogenetic null modelling approaches were combined with pollinator interaction networks to elucidate the processes structuring flower colour assembly patterns in Asteraceae communities in Namaqualand, South Africa.

Plant species were assigned to flower colour pattern categories (CPCs) that incorporate the complexity of the bulls-eye colour pattern, using pollinator vision models. Null models were employed to assess whether daisy communities exhibit clustering (driven by filtering, facilitation, or convergence) or overdispersion (driven by competitive exclusion or character displacement) of CPCs. Next, flower visitor networks were constructed for communities with non-random CPC assembly to confirm the functional role of pollinators in determining floral trait assembly.

Plant species are unevenly distributed across CPCs, the majority of which are not phylogenetically conserved, suggesting that certain CPCs have a selective advantage. Clustering of CPCs in communities is more frequent than overdispersion, and this does not reflect non-random phylogenetic assembly. In most communities at least one CPC is overrepresented relative to null assemblages. Interaction networks show that each community has a single dominant pollinator that strongly interacts with the overrepresented CPC, suggesting a role for pollinator preferences in driving clustered assembly of CPCs within daisy communities.

This novel approach, that demonstrates non-random assembly of complex flower colour patterns and corroborates their functional association with particular pollinators, provides strong evidence that pollinators influence plant community assembly. Results suggest that in some community contexts the benefits of pollinator sharing outweigh the costs of

heterospecific pollen transfer, generating clustered assembly. They also challenge the perception of generalised pollination in daisies, suggesting instead that complex daisy colour patterns represent a pollination syndrome trait linked to specific fly pollinators.

Introduction

Studies of plant community assembly usually consider interactions between plants and their abiotic environments, highlighting the importance of species filtering by abiotic environments (e.g. climate, soils – Kraft *et al.* 2015) and competition for limited resources (light, water, nutrients - Tilman 1994, 2004; Rees et al. 2001) in structuring plant communities. However, seed set, and thus persistence of a species in a community, is dependent on successful pollinator-mediated fertilization in the vast majority of angiosperms (Ollerton *et al.* 2011), and this is particularly true for annual species. Thus pollinators represent a necessary resource for plant reproduction, and we would expect ecological sorting, and the evolution of particular suites of functional (pollination-linked) traits, to result from interactions between plants for the pollinator resource. In addition, the available pollinator fauna (i.e. the pollinator climate – Grant and Grant 1965) might impose strong filters on which plant species can successfully occur in communities. Because floral traits are largely shaped by pollinator-mediated selection, and less so by abiotic conditions (reviewed in Harder and Johnson 2009), the importance of pollination interactions in plant community assembly should be evident in the way floral traits are arrayed across species within communities relative to the regional species pool (Sargent and Ackerly 2008). A substantial body of work, focused on the structuring of flowering phenologies within communities in response to competition mediated through heterospecific pollen transfer, has provided mixed evidence for the importance of pollination in plant community assembly (e.g. Poole and Rathcke 1979; Rabinowitz *et al.* 1981; Rathcke 1983; Armbruster 1986; Moeller 2004; Aizen and Vazquez 2006). However, the assembly of floral traits that function in both pollinator attraction (e.g. colour pattern, scent, reward type) and the efficiency of the pollination process (e.g. mechanical fit traits) has received less attention (but see Muchhala and Potts 2007; McEwen and Vamosi 2010; Heystek and Pauw 2014; Muchhala *et al.* 2014; Runquist *et al.*

2016). These are traits that can influence both the pollination niche of a plant species, and its interactions with other plant species vying for the available pollinator resources.

Competition for pollinators is likely to lead to overdispersion of floral traits within communities (Fig. 2.1). This can occur through direct competition for pollinator visits where some flowers are more attractive to particular pollinators than others (Rathcke 1983; Brown *et al.* 2002; Mitchell *et al.* 2009), or through indirect competition mediated by the fitness costs of heterospecific pollen transfer (HPT) between plants that share pollinators (Waser 1978; Cambell and Motten 1985; Fishman and Wyatt 1999; Mitchell *et al.* 2009; Matsumoto et al. 2010). Both competition pathways could lead to competitive exclusion of weaker competitors from communities, or alternatively, they could drive the evolution of character displacement and consequent divergence of pollination niches between community members (e.g. Muchhala and Potts 2007; Morales and Traveset 2008; Grossenbacher and Stanton 2014). It is often difficult to discern whether competitive exclusion or character displacement is driving trait overdispersion, but if character displacement is occurring, trait overdispersion and shifts between pollinators is likely to be more pronounced in sympatric populations than in allopatric populations (Coyne & Orr 1989).

A clustered distribution of floral traits within communities could result when pollinator climates vary spatially across the landscape and the benefits of shared attraction outweigh the costs of HPT (Fig. 2.1). Clustering of floral traits, such as flower colour pattern, within communities relative to their distribution in the regional species pool also implies spatial structuring of traits across the region; where, for instance, the dominant flower colour pattern will vary between communities. Plant species might be excluded from communities if their pollinators are absent, in a process akin to environmental filtering. Trait clustering within communities could also be caused by contemporary facilitative interactions, where an

increase in the number of plant species with the same floral phenotype increases pollinator visitation rates, either by providing a more prominent attraction signal (Ashton *et al.* 1988) or by providing a more varied resource base (Ghazoul 2006). Alternatively, selection imposed by spatially variable dominant pollinators can lead to evolutionary convergence of floral traits amongst community members. The limited previous work using null models to explore floral trait distributions within communities has mostly shown support for overdispersion in pollination traits (Muchhala and Potts 2007; McEwen and Vamosi 2010; Heystek and Pauw 2014; Muchhala *et al.* 2014; Runquist *et al.* 2016), but limited support has also been found for clustering of traits within communities (De Jager et al. 2011; Runquist et al. 2016).

In addition to producing distinctive patterns of floral trait distributions within communities, mechanisms of pollinator-mediated community assembly should also create clear signal in the structure of community plant-pollinator interaction networks. For example, if communities exhibit overdispersed assembly of floral phenotype, suggesting character displacement or competitive exclusion as likely structuring mechanisms, we would expect interaction networks to comprise multiple distinct modules, each representing subsets of plant and pollinator species interacting more strongly with each other than with other subsets in the network. In contrast, if community assembly models show clustering of floral traits, where filtering, facilitation or convergence is inferred, we expect interaction networks to be dominated by a single, or few, functional pollinator types that strongly interact with plants possessing the over-represented floral traits to form a single large module.

Here we assess pollinator-mediated assembly of daisy (Asteraceae) communities in the Namaqualand subregion of South Africa. This system is characterised by a narrow temporal flowering window, high densities of flowers, low incidence of selfing and the predominance of annual species, factors that are likely to increase reliance on seed-mediated reproductive

assurance and the importance of pollination interactions (De Waal et al. 2014). In addition, exceptionally high angiosperm diversity (~ 3,800 angiosperm species, and > 400 species of Asteraceae, in ~ 55,000 km^2; Snijman 2013) provides a rich regional species pool, ideal for testing community assembly hypotheses. Every year in spring, mass flowering displays, dominated by daisies, carpet the Namaqualand landscape in swathes of colour. Our observations, and anecdotal reports, suggest that dominant daisy flower colours vary across the region with, for example, white colours dominating on the sandy coastal plain and orange predominating in the upland areas (e.g. Fig. 2.2). Recent work on other Meditteranean-type ecosystems has shown that flower colour mediates facilitative interactions, and that both flower colour and scent structure plant-pollinator networks (Kantsa *et al.* 2017). The daisy 'flower' is a compound inflorescence (the capitulum) with an outer ring of petal-like ray florets, that are frequently bi-coloured, surrounding an inner circle of disc florets. This capitulum arrangement produces a complex bulls-eye colour pattern, often comprising three distinctly coloured concentric rings. The open shape of the capitulum does not physically preclude visits from any insects, nor pollination by them, and daisies are thus widely considered to be pollination generalists (Fenster *et al*. 2004). However, like other plants with apparently generalist floral phenotypes, daisy species may exploit only a subset of the available pollinator species within a community (e.g. Ellis and Johnson 2009; de Jager and Ellis 2014; de Waal *et al*. 2015). One possibility is that distinct daisy colour patterns (i.e. different combinations of contrasting concentric colour rings) represent different floral syndromes, or traits associated with distinct groups of flower visiting animals (Fenster *et al.* 2004). In some cases flower colour, and the pattern of colour arrangement, have been shown to strongly influence both pollinator preference (Bradshaw and Schemske 2003; Horth *et al.* 2014) and pollinator constancy (Hill *et al.* 1997), consequently influencing pollination success (Johnson and Dafni 1998a; Goodale *et al.* 2014; Koski and Ashman 2014, 2015).

While some studies have considered how flower colours are assembled in communities (McEwen and Vamosi 2010; De Jager *et al.* 2011; Muchhala *et al.* 2014), none have investigated the assembly of more complex colour patterns. This perhaps reflects the difficulty of considering complex phenotypes such as the typical bulls-eye colour pattern of radiate daisy capitula (e.g. Fig. 2.3).

We first used an objective categorisation scheme based on reflectance measurements from ray and disc florets to classify each species in the regional pool of Namaqualand daisies into a colour pattern category (hereafter CPC), providing a measure of pattern diversity. This was done based on raw reflectance spectra and accounting for the visual systems of the dominant pollinators in the system (bees and flies). We then built a genus-level phylogeny of the sampled species to assess phylogenetic signal of flower colour patterns and to determine whether communities exhibit significant phylogenetic structuring. Next we used null models to investigate how colour patterns are assembled in communities. We expected CPCs to be overdispersed in communities if pollinator-mediated competitive exclusion or character displacement structures communities (Fig. 2.1). Alternatively, if pollinator-mediated facilitation, filtering or convergence structure communities we expected that the majority of plant species should represent just a few CPCs within a community (i.e. clustering of CPCs), and that the dominant CPCs should vary between communities across the region. Next, we assembled multiple pollinator visitation networks to determine whether network structure conforms to expectations based on the processes identified by the community assembly models. Finally, in order to confirm the functional role of CPCs in determining the pollination niche, we assessed the influence of flower colour pattern on plant-pollinator interaction strengths in the visitation networks.

Methods and materials

Study system

Limited work on the pollination systems of Namaqualand daisies suggests pollinator abundances are low despite the extremely high densities of rewarding flowers (Struck 1994). Flower visits are dominated by bees (Apoidea), flies (Bombyliidae, Tabanidae) and Hopliini beetles (Scarabaeidae), and pollinator assemblage composition varies at fine spatial scales (Struck 1994; Ellis and Johnson 2009). Further work has shown pollinator preferences for particular flower colour patterns that result in divergent selection on floral phenotype (Ellis and Johnson 2010; de Jager and Ellis 2012, 2014, but see Ellis and Johnson 2012).

Sampling

Plant community composition and flower spectral reflectance data

Plant species occurrence data and floral reflectance spectra were collected for all annual and perennial daisy species (and colour forms of polymorphic species) at sixteen sites (0.5 x 0.5 km) in Namaqualand during the last week of August and first week of September 2007, and at four additional sites during in 2015 [Appendix 1 Table S2.1]. Sites were located 2.8 – 282.8 km apart (mean ± sd = 81.7 ± 55.3 km). Spectra were recorded indoors at a 45° angle to the floret surface using an OceanOptics USB4000 Spectrometer calibrated with a diffuse reflectance WS-2 white standard. Spectra were averaged over three measurements of flowers from different plants chosen at random at a site. To quantify flower colour pattern, which in daisies conforms to a series of concentric circles, three points on the capitulum of each species were sampled (Fig. 2.3): (1) the outer ray floret (OR – Fig. 2.3A), (2) the inner ray floret (IR – Fig. 2.3B), and (3) the disc floret petals (D – Fig. 2.3C). It was not always

possible to accurately measure reflectance of disc florets due to their small size and changeable orientation of disc floret corolla lobes. However, across species disc spectra always conformed to either a yellow or black colouration. Thus, in order to reduce the effects of disc measurement inaccuracy on our model results, the 'D' measure in all species was assigned either a yellow or a black spectrum.

Interaction networks

Flower visitor networks were constructed in August and September 2015 for two of the sampled community types that showed non-random assembly patterns. Networks were sampled in the same community types as represented by community 2 and community 7, but not at the exact localities, and in a different sampling year. Three 100 x 100 m sites were sampled within each of the two community types, to produce three networks in each community type. Flower visitors to all daisy species were recorded during 15 minutes observation periods in 100 1 m^2 plots at each site, resulting in 25 observation hours per site and 75 observation hours per community type. For each plot, the number of open flowers of each plant species was recorded and the number of visits to each plant species from each insect species was noted. Insect visitors were caught, preserved and sorted to morphospecies.

Phylogeny inference

Our Namaqualand daisy communities incorporated 76 species from 27 genera. Sequence data were obtained from Genbank for representatives of each genus [Appendix 1 Table S2.2] for two nuclear (ETS and ITS) and four plastid DNA regions (*matK*, *ndhF*, *rbcL* and the *trnL-trnF* region). Although some gene regions were lacking for some genera, most (22 of 27) were represented by four or five regions. We estimated a genus-level phylogeny using a

rooted phylogenetic approach in the BEAST v. 1.8.3 software (Drummond *et al.* 2012). See supplementary materials for a detailed description of phylogeny estimation.

Data analysis

Identifying flower colour pattern categories (CPCs)

All analyses were performed in R (R Core Team 2016). Sampled species/colour forms were classified into CPCs using three approaches. First we followed the approach of Shrestha *et al*. (2014) to identify CPCs from raw reflectance spectra. We then incorporated visual models of bees (Chittka 1992) and flies (Troje 1993).

Raw reflectance spectra:

Colour of each measured part of the capitula (OR, IR, D) was categorised by identifying inflection points along raw reflectance spectra (i.e. points where the direction of the curvature of the spectrum changes), after lumping reflectance values into 1 nm bins (see Shrestha *et al.* 2014). Eight 50 nm windows across the UV/VIS spectrum (i.e. 300 – 700 nm) were examined for the presence of inflection points. Changes from a concave-downward to a concave-upward curve (i.e. decreasing reflectance) were scored as negative inflection points, while the opposite changes were scored as positive. Each 50 nm window was scored as having no inflection point (0), a negative inflection point (-1), or a positive inflection point (1) (see Fig. 2.3). These eight variables were then used to group raw reflectance spectra in a cluster analysis using Euclidean distances in the R 'pvclust' package (Suzuki and Shimodaira 2015). Clustering was performed separately for reflectance spectra from the OR and IR sections of the capitulum, and plant species showing at least 90% similarity were grouped together in a

cluster. As D sections were assigned only to black or yellow, no clustering was necessary. Next, plant species belonging to the same combination of OR, IR and D clusters were grouped together as a CPC. Species lacking ray florets were all placed in a single CPC, as these were all found to have yellow disc florets.

Bee vision model:

A separate group of CPCs were identified based on how they are perceived by honeybees using Chittka's (1992) honeybee visual model. Since this model requires a background spectrum for contrast, we used a standard green spectrum as background for the OR reflectance spectra, the OR spectra as background for the IR spectra, and the IR spectra as background for the D spectra. The x and y coordinates of the hexagon plot for each of the three flower sections were used to cluster species using Euclidean distances in 'pvclust' (Suzuki and Shimodaira 2015). Plant species showing 70% or greater similarity were grouped together, as we found that this threshold divided plant species into intuitive groups, while minimising the number of groups that contained only a single species across the regional pool. We refer to the groupings as bee CPCs.

Fly vision model:

To categorise the colour patterns according to fly visual perception, we used Troje's (1993) fly visual model in the 'pavo' package (Maia *et al.* 2016). As in our bee vision analysis, we used standard green as background for the OR, the OR as background for the IR, and the IR as background for the D spectra. This fly vision model does not encompass gradients of colour, but packages colours into four discrete categories distinguishable by flies, to which we assigned each flower colour measurement. Plant species with the same combination of

categories for the three floral sections were grouped into the same CPC phenotype, and we refer to these as fly CPCs.

Phylogenetic signal in CPCs and in community composition:

To assess whether variation in colour pattern is significantly affected by common ancestry, we examined the correlation between evolutionary divergence (as measured by the branch lengths in our BEAST MCC tree [Appendix 1 Fig. S2.1]) and colour pattern variation between daisy species in our sampled regional pool. A Brownian motion model of trait evolution was used to estimate the value of Pagel's λ (Pagel 1999) in the 'phylosignal' package (Keck 2015). As the CPC phenotype is unordered and not continuous, we tested for phylogenetic signal in each CPC separately, treating each as a binary variable with species either belonging to it, or not. If colour pattern is a pollination syndrome trait, we would expect repeated evolution of colour pattern categories across the phylogeny; alternatively, if it is not under selection or is phylogenetically constrained we expect the categories to exhibit strong correlation with phylogenetic relatedness.

We also tested for phylogenetic structuring of plant community composition, because non-random assembly of CPCs in communities might reflect non-random phylogenetic composition of plant communities if CPCs are phylogenetically conserved. Here we used the 'phylostruct' function in the 'picante' package (Kembel *et al.* 2010) with Gotelli's independent swap algorithm (Gotelli 2000).

Community assembly patterns

Community assembly patterns were characterised in a stepwise manner. First, we performed 999 random assemblies of species into communities from the regional pool using Patefield's

(1981) r2d null model algorithm, keeping both row and column totals constant. For each of the randomly assembled communities, plant species were replaced by their CPC (this was repeated for categories derived from raw spectra, from bee visual space, and from fly visual space). The polymorphic plant species (those exhibiting multiple CPCs that always occurred allopatrically) were randomly represented by one of their observed CPCs. A community-level CPC diversity metric was calculated for each randomly assembled community to assess whether communities show overdispersion or clustering of CPCs. Community CPC diversity was quantified using a Hill number adjusted Sørenson alpha diversity index (Jost 2007), which incorporates the evenness of the distribution of plant species across CPCs. For example, if many plant species share a colour pattern, then the effective number of CPCs (i.e. CPC diversity) is lower. Observed CPC diversity was calculated for each of the twenty sampled communities separately, and then compared to null communities to test for departure of the metric from expectations under random assembly. Significantly lower observed CPC diversity within a community would indicate clustering, while significantly higher values indicate overdispersion.

We further examined whether assembly of species from the regional pool into local communities was non-random by computing a phenotype-level metric using z-scores to determine whether the observed number of plant species belonging to each CPC in each community differed from the random expectation. This phenotype-level metric explicitly tests for the pattern underlying CPC clustering at the community level, i.e. over-representation of individual CPCs within communities. As the test is sensitive to the presence of regionally rare CPCs within communities we adopted a conservative approach and only computed this metric for CPCs represented by ten or more taxa in the regional pool (8 of 28 raw CPCs, 8 of 21 bee CPCs, 5 of 8 fly CPCs). Thus the test asks whether the regionally most common CPCs are overrepresented in local communities relative to the regional pool, as

would be expected under assembly through facilitation or convergence driven by spatial variation in dominance of pollinators with different colour preferences.

Interaction network patterns

If community assembly patterns result from competitive exclusion or character displacement (i.e. colour patterns are overdispersed), we expect strongly modular networks, with evenly sized compartments centred on many different insect species. We explored this expectation in two ways. First, we calculated modularity for each network using the QuanBiMod algorithm (Dormann and Strauss 2014), and compared observed values to 999 randomised values to assess significance. Next, we tested the expectation of an even spread of interactions across many insect visitor species using rank-abundance approaches from community ecology. Flower visiting species were first ranked, in descending order, on the basis of their interaction frequencies in order to produce rank-abundance (or rank-interaction frequency) curves for the overall networks for each community type. If interaction frequencies are evenly spread across pollinator species, then a broken-stick distribution (MacArthur 1957) is expected, while a Zipf distribution (Zipf 1949) (characterised by few elements that occur very frequently and many that occur rarely) is expected if interactions in networks are dominated by one (or few) pollinator species. We compared the fit of these two distributions to our data for the two community types separately using AIC values computed by the 'radfit' function in 'vegan'.

If community assembly patterns result from filtering, facilitation or convergence (i.e. colour patterns are clustered), we expect to find a single (or few) dominant pollinator(s) that strongly interacts with the dominant floral phenotypes. To test this we calculated the link temperature (see Junker *et al*. 2010) of the interaction between the dominant pollinator species and each plant species in each community. Link temperature ranges from -1 to 1, where positive values

indicate disproportionately favoured interactions and negative values indicate avoided interactions. We used ANOVA with *a priori* contrasts to assess whether the overrepresented CPCs (identified in the null model section) in these two communities are associated with high link temperatures to the dominant pollinator. This was done separately for the two communities, and separately for CPCs based on raw spectra, bee vision and fly vision.

Results

Floral colour pattern categories (CPCs)

We recorded 76 daisy species from 27 genera at our twenty sites. Community plant species richness ranged from 3 to 25 (mean = 12.9). Plant species were assigned to 28 CPCs based on inflection points from raw reflectance spectra, and the number of CPCs in each community varied from 2 to 16 (mean = 8.8) [Appendix 1Table S2.3]. Eight species were polymorphic (based on raw spectra), i.e. comprised more than one CPC. Colour patterns varied in the number of plant species assigned to them (range = 1 – 11; mean = 3.21), and the five largest CPCs (each containing 4-8 genera) contained 51% of plant species. Most plant species thus tend to belong to just a few CPCs. Seven of the 28 CPCs (i.e. 25 %) exhibited significant phylogenetic signal [Appendix 1 Table S2.4].

Based on bee visual space, plant species were assigned to 21 CPCs, which again varied in the number of plant species they contained (range = 1 – 12; mean = 4.29), and the five most common bee CPCs contained 60% of plant species. Each community harboured between 2 and 12 bee CPCs (mean = 7.7). Four (19 %) of the bee colour patterns exhibited phylogenetic conservedness. Only eight CPCs were identified based on fly vision (size range = 1 – 51

species; mean = 11.25), and two fly CPCs contained 71% of the plant species. The three most common fly colour patterns (37.5 %) exhibited significant phylogenetic conservedness.

Communities exhibited random phylogenetic assembly patterns ($p > 0.05$), and thus community assembly patterns are independent of phylogeny.

Community assembly patterns

In more than half of the communities, at least one CPC was overrepresented relative to its occurrence across the landscape as a whole (the phenotype-level metric in Table 2.1). This pattern held regardless of whether colour patterns were delineated using fly, bee or no visual model. Clustering was the most common assembly pattern detected using the CPC alpha diversity metric. Only two communities exhibited significant overdispersion for this metric (communities 17 and 19, Table 2.1), while eight communities exhibited significant clustering of colour pattern. However, in the majority of communities, we detected no departure from random assembly for the three methods of pattern delineation for the alpha diversity metric.

Network patterns

Three interaction networks from three separate sites were constructed for each of the two examined community types. Community type 2 contained 19 daisy species, 109 insect morphospecies, and 7811 interactions while community type 7 contained 12 daisy species, 47 insect morphospecies and 3161 interactions. All six networks from both communities were less modular than expected by chance ($Q_{mean} = 0.26$; $SD = 0.11$; $z_{mean} = -4.50$; $p < 0.05$), thus providing support for neither character displacement nor competitive exclusion.

For Community 2, interaction frequencies were unevenly distributed across pollinator species ($AIC_{Zipf} = 1101$, $AIC_{broken\text{-}stick} = 21600$). The beefly *Megapalpus capensis* was responsible for

68% of interactions in community type 2 (Fig. 2.4). For Community type 7, interaction frequencies were also unevenly distributed across species (AIC_{Zipf} = 323, $AIC_{broken\text{-}stick}$ = 6217) and a single horsefly (*Rhigioglossa* sp.) species contributed 57% of interactions. Analysis of variance with *a priori* contrasts showed that overrepresented CPCs were significantly associated with high link temperatures to the dominant pollinators in both community types (Table 2.2; Fig. 2.4).

Discussion

Our analysis demonstrates clustered assembly of flower colour patterns in Namaqualand daisy communities, confirming anecdotal observations that the dominant colour patterns in communities vary across the landscape. By drawing on additional insights from interaction networks, we provide direct evidence that spatially variable pollinator climates underlie this clustered assembly pattern.

Community assembly through filtering, ecological facilitation, or evolutionary convergence

Sixteen of twenty sampled communities contained at least one regionally common CPC that was overrepresented within the community relative to the regional pool, and the community-level CPC diversity metric suggest clustering more frequently than overdispersion (although in most cases it suggests random assembly of colour pattern). This result is consistent with facilitation or evolutionary convergence driving coexistence of species with a shared colour pattern favoured by locally dominant pollinators, and suggests that competition for pollination is not a dominant process influencing daisy community assembly. Further, while we have not incorporated relative density of individual plant species within each community, accounting for this would likely strengthen the pattern we discern, i.e. overrepresented colour

groups within communities usually encompass the most common species in communities (Fig. 2.2).

Clustering of flower colour pattern within communities could arise through several mechanisms. First, we would expect clustering if colour pattern is strongly phylogenetically conserved and strong environmental filters (pollinators or other abiotic filters) result in frequent coexistence of related taxa with similar traits (i.e. phylogenetically clustered community assembly – Wolowski *et al.* 2017). We can reject this possibility as we find no evidence for non-random community assembly in terms of phylogeny, and limited evidence for phylogenetic conservatism of colour pattern.

Second, abiotic filters could account for the clustering of flower colour. Indeed, several studies have demonstrated an influence of environmental factors, such as incident UV light, on distribution of colour pattern. Koski & Ashman (2014), for example, showed a global latitudinal cline in the UV-bullseye pattern, where the bullseye is larger in regions closer to the Equator. Warren & Mackenzie (2001) showed that more pigmented floral forms have higher fitness under drought stress, which could lead to clustering of flower colours across rainfall gradients. While we cannot exclude this possibility, it seems unlikely that abiotic factors are the primary determinants of flower colour pattern in our system, as our sampling area was relatively small (maximum distance between two sites was 282.8 km) and does not span a large range of altitude, latitude or climatic variability. Also, many colour patterns spanned the whole sampling area, varying only in frequency between communities.

Third, pollinators can drive the clustering of flower colour pattern in communities through three distinct mechanisms: 1) ecological filtering might exclude colour patterns mismatched with local pollinator preferences, 2) positive facilitative pollinator-mediated plant-plant interactions would favour recruitment into communities of colour patterns that are already

present, and 3) selection by dominant pollinators could result in evolutionary convergence of species on preferred colour patterns. We interpret our results as indicating that the clustered distribution of colour pattern that we observe within communities relative to null assemblages is due to these mechanisms operating across a geographic mosaic of dominant pollinators, i.e. when dominant pollinators with varying colour preferences differ between communities. This interpretation is supported by visitation network data that demonstrate the existence of a geographic mosaic of dominant pollinators and a functional link between colour pattern and the dominant pollinators present in communities.

The beefly *Megapalpus capensis* and the horsefly *Rhigioglossa* sp., respectively, were dominant in the two community types for which interaction networks were constructed. For both of these pollinators, interaction strengths were strongly influenced by floral CPC, suggesting that this trait may be under selection by pollinators. In both communities, the dominant pollinators interacted most strongly with colour patterns that were overrepresented in these communities. Although pollinator interaction frequency is not necessarily a proxy for pollinator effectiveness, it is likely an important determinant of pollinator efficiency in daisies where the open capitulum structure ensures that all visitors can remove and deposit pollen. Interestingly, the switch between two dominant pollinators across community types was a switch between two fly species, which would often be classified into the same functional group. *Megapalpus capensis* is known to choose flowers based on visual signals and to prefer orange flowers with black centres (De Jager and Ellis 2012, 2013), but experimental evidence for colour preferences of other fly species in this region is unfortunately lacking. Particularly, Troje's (1993) fly visual model might not fully capture the visual discrimination capabilities of the pollinators in this system. Fly vision, particularly the range of variation in colour perception between species, is not fully understood. Although colour vision has been shown for all fly species tested to date, it is unclear whether all species

have categorical vision (reviewed in Lunau (2014), and different species might have different spectral tuning filters that can alter what flies see (Lunau and Knüttel 1995). Recent work on bees has shown that caution should also be applied when interpreting bee colour vision models, as bees' colour discrimination abilities are potentially influenced by neural tuning (Avarguès-Weber and Giurfa 2014), and can vary between bee species (Garcia *et al.* 2017). Although colour discrimination modelling can be done using models of closely related species, ideally models should always be verified with behavioural data (Garcia *et al.* 2017), which did not fall within the scope of our study.

While our data suggest a strong influence of pollinators on colour pattern assembly, we cannot unequivocally separate the influence of the three possible mechanisms that could result in clustering of colour pattern within communities (Fig. 2.1). However, our results suggest that the clustering of flower colour patterns is, at least partly, driven by evolutionary convergence on pollinator-preferred patterns. Most daisy species belong to just a few colour pattern categories that have evolved repeatedly in several distantly-related daisy lineages, a pattern likely resulting from convergence through pollinator driven selection. Also, eight of our sampled plant species exhibited allopatric intraspecific CPC polymorphisms, again demonstrating the evolutionary lability of flower colour in these daisies. Flower colour patterns may thus represent independent pollinator syndromes generated by pollinator-driven convergence, where syndromes are specific to particular functional groups of pollinators, or even to specific pollinator species. Flower colour is frequently an important syndrome trait (Fenster *et al*. 2004; but see Reverté *et al.* 2016), which in some cases is associated with a single pollinating species, as in the case of the long proboscid fly, *Prosoeca longipennis,* that visits 17 plant species in South Africa, all of which exhibit matching long-tubed flowers and similarly-coloured petals (Newman *et al.* 2014). Similarly, our results suggest that the differential colour pattern preferences of *Rhigioglossa* sp. and *Megapalpus capensis* may be

generating syndromes of floral traits in daisy species. Previous work has shown that fly pollinators have different innate colour preferences than bees (Shrestha *et al.* 2016), and that bees also exhibit differential colour preferences (Dyer *et al*. 2016).

Although we cannot unequivocally rule out filtering as a process, the presence of many different floral phenotypes in each community suggests that strong filters for single or just a few flower colour patterns are not present. This may indicate that most pollinator types are present in all communities, but that pollinator abundances vary strongly across the region. Evidence for filtering of plant species by pollinator availability exists in the invasion and agricultural literature (Blanche *et al.* 2006), but has not been demonstrated extensively in natural systems (Sargent and Ackerly 2008). In particular, filtering seems unlikely in systems where plants have easily accessible floral rewards, such as daisies, and are not in specialised mutualisms with just one or a few species of pollinators. However, certain flower colours could be filtered from communities if the available pollinators are unable to detect them. For instance, Bukovac *et al.* (2017a) recently showed that flowers with single inflection points between 420 and 480 nm are not easily detected by bees, and that these colours are rarely found in flowers.

While several studies have demonstrated facilitative pollination interactions in other floras (e.g. Tur *et al.* 2016), the only existing study in Namaqualand daisy communities found no evidence for facilitation of visitation (De Waal *et al.* 2015). Facilitation is most likely at low plant densities (Moeller 2004; Ghazoul 2006), while daisy flower densities in the Namaqualand spring mass flowering events are often very high (Fig. 2.2). However, it is possible that facilitation may promote infiltration of species into communities with colour patterns that are already present, as new immigrants to a community will always initially occur at lower densities (as argued by De Jager *et al.* 2011).

Plant community assembly through competition for pollinator resources or character displacement

If competition for pollinators is an important mechanism structuring plant communities, we expected to detect both overdispersion of colour patterns within communities, and strongly modular interaction networks. Both of these patterns are consistent with pollinator niche divergence of community members. We found very little evidence of these patterns, as colour pattern overdispersion was only detected in two communities, and interaction webs were not significantly modular. In addition, both of the overdispersed communities were relatively species-poor, and contained regionally rare colour patterns that were overrepresented in these communities relative to the regional species pool, which may account for the overdispersed pattern.

In this sense our findings contrast with most previous studies of floral trait assembly, which show overdispersion patterns indicative of either character displacement or competitive exclusion (Muchhala and Potts 2007; McEwen and Vamosi 2010; Heystek and Pauw 2014; Muchhala, Sönke Johnsen, *et al.* 2014), although one previous study has demonstrated clustering of flower colour within *Oxalis* communities (De Jager *et al*. 2011). One possibility is that floral community trait assembly reflects a system-specific balance between the costs of heterospecific pollen transfer and the benefits of sharing pollinators. Tur *et al.* (2016), for example, showed that although south Andean plants share pollinators, the costs of HPT do not outweigh the benefits of facilitation, thus making clustered floral trait assembly likely. Because of the strong dominance of a single pollinator species in our daisy interaction networks, the benefits of exploiting the dominant pollinator (i.e. high visitation rates) may well outweigh the HPT costs of sharing it with co-occurring plant species.

Conclusions

The clustered assembly of complex flower colour patterns we demonstrate adds to the accumulating evidence that pollinators are an important determinant of plant community assembly. Our novel approach, of combining a standard community trait and phylogenetic null modelling approach with insights from pollination interaction networks, goes beyond previous studies in providing direct evidence for the role of pollinators in driving floral trait assembly patterns. We show that pollinator mediated assembly need not involve competitive interactions. Instead in some ecological contexts, such as when pollinator communities are dominated by one or few species as was the case here, the benefits of pollinator sharing might outweigh the costs of competition to generate clustered assembly. In addition, our findings are particularly interesting given the prevailing perception of the generalised pollination systems of daisies, suggesting instead that daisy colour patterns represent a pollination syndrome trait linked not only to specific pollinator functional groups, but also to different species within a functional group (e.g. different species of fly).

Tables

Table 2.1. The community structuring of complex flower colour patterns (categorized using raw spectra, bee vision and fly vision models) across twenty daisy communities from Namaqualand, South Africa. Non-random assembly of colour patterns (CPCs), detected using community (CPC diversity) and phenotype-level metrics, is indicated for each community. "C" indicates significant phenotypic clustering ($p < 0.05$); "O" indicates significant phenotypic overdispersion; blank cells represent random assembly. Values that are nearly significant ($p < 0.07$) are also indicated (*). The number of species and CPCs (n) are indicated for each community. The last two rows provide the percentage of either clustered (C) or overdispersed (O) communities according to each measureValues that are nearly significance ($p < 0.07$) are also indicated (*).

		Raw spectra			Fly vision			Bee vision		
Community	Number of species	n	Alpha diversity	Phenotype-level	n	Alpha diversity	Phenotype-level	n	Alpha diversity	Phenotype-level
1	9	6	C*	C	4			7		
2	13	11		C	4	C*	C	9		
3	15	9		C	5		C	10		
4	23	12	C	C	4	C	C	12		C
5	12	9		C	4		C	8		C
6	8	7			3	C*		6		
7	15	8	C	C	6	C*	C	8		C

8	8	7			4			7			
9	25	12	C*		6		C	9	C		
10	12	10			4		C	8			C
11	14	11		C	4		C	9			C
12	12	9		C	4			8			C
13	18	12		C	6		C	9	C		C
14	18	12		C	5		C	9			C
15	25	16		C	6		C	12			C
16	16	12			6			10			C
17	3	3	O		1		C	3	O		
18	5	4			3		C	4			
19	4	4	O		3			4	O		
20	3	2			2			2			
% clustered			20	55		20	65		10	50	
% overdispersed			10	0		0	0		10	0	

Table 2.2. The effect of colour pattern categories (CPCs), delineated from raw spectra, bee vision and fly vision, on link temperature of the dominant pollinator species in two clustered communities. The effects of overrepresented colour pattern categories were tested with ANOVA with *a priori* contrasts.

		Community 2 *Megapalpus capensis*			Community 7 *Rhigioglossa* sp.		
		d.f.	F	p	d.f.	F	p
Colour pattern category	Raw spectra	**9, 15**	**3.271**	**0.02**	**8, 15**	**3.634**	**0.02**
	Bee vision	**8, 16**	**3.64**	**0.01**	**7, 16**	**2.745**	**0.04**
	Fly vision	3, 21	1.274	0.31	5, 18	1.403	0.27

Figures

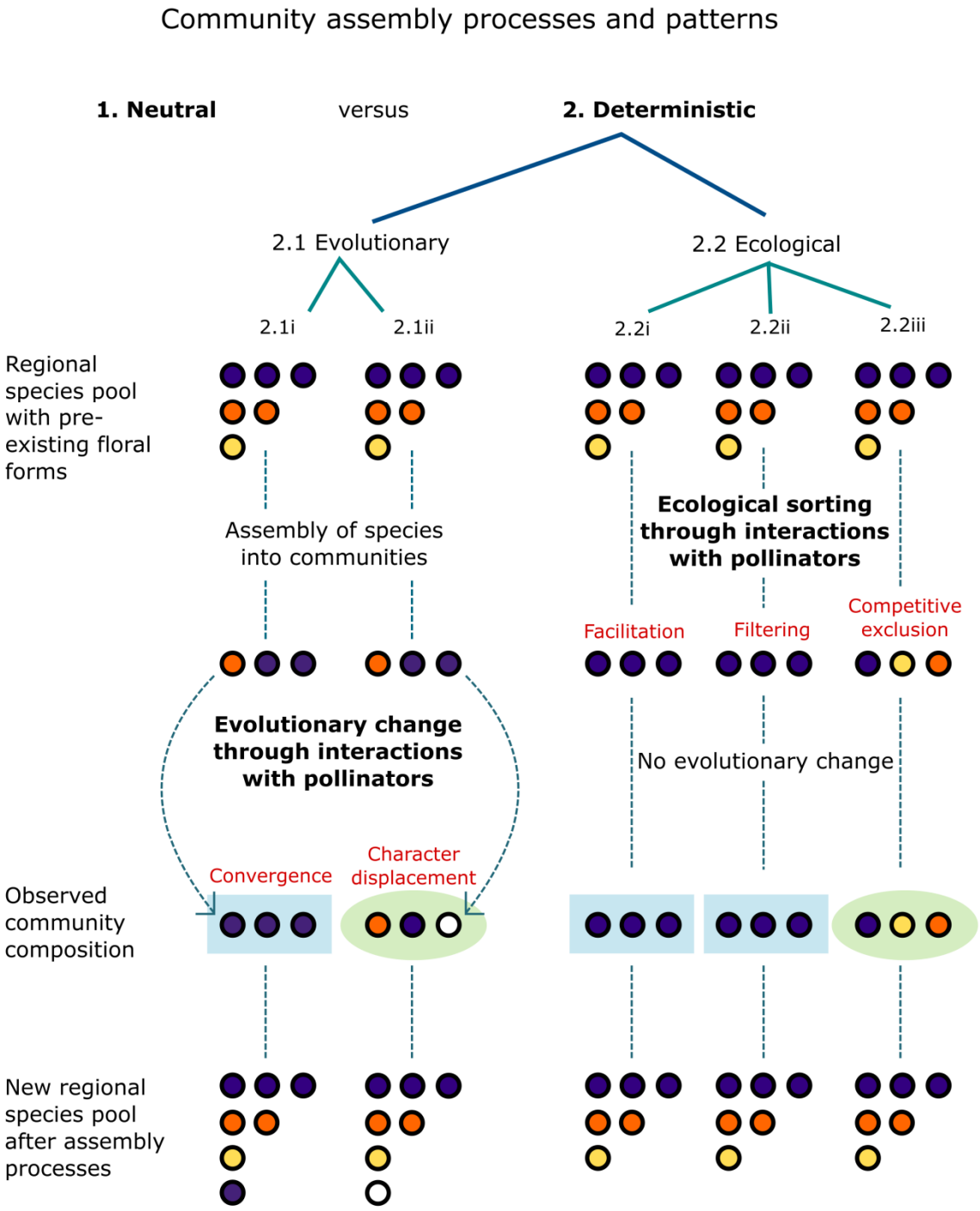

Figure 2.1. The assembly of plant traits into communities from a regional pool occurs through either (1) random or (2) deterministic processes. Deterministic processes can either be (2.1)

evolutionary, where traits evolve in response to selection within communities, or (2.2) ecological, where ecological sorting of species into communities depends on their pre-existing traits. If pollinators have visual preferences, then selection may favour certain flower colours over others, leading to convergent flower colour evolution and clustering of flower colour within a community (2.1i). Similarly, flower colours within a community may evolve to be different from one another if there are costs associated with pollinator sharing (2.1ii). Alternately, pre-existing flower colours may be sorted into communities by ecological interactions (2.2). Plant species may facilitate the recruitment of other plant species with similar flower colours into a community if a pollinator species with strong preferences performs better with a varied resource base (2.2i). Strong pollinator preferences can also act as a filter to plant species that have a non-preferred flower colour (2.2ii). Competition between plant species for the available pollinator resources can lead to similar flower colours failing to recruit into a community, which leads to a variety of flower colours in the community. Thus, convergence, facilitation, and filtering will lead to clustered trait assembly patterns, and character displacement and competitive exclusion will lead to trait overdispersion.

Figure 2.2. The semi-desert Namaqualand sub-region of the Succulent Karoo in South Africa transforms annually during a mass flowering display in the austral spring. The display is dominated by daisies, most of which are self-incompatible annuals that are reliant on pollinators for seed set and population persistence. The photos illustrate changes in dominant flower colours across communities. Photos by JEK and AGE.

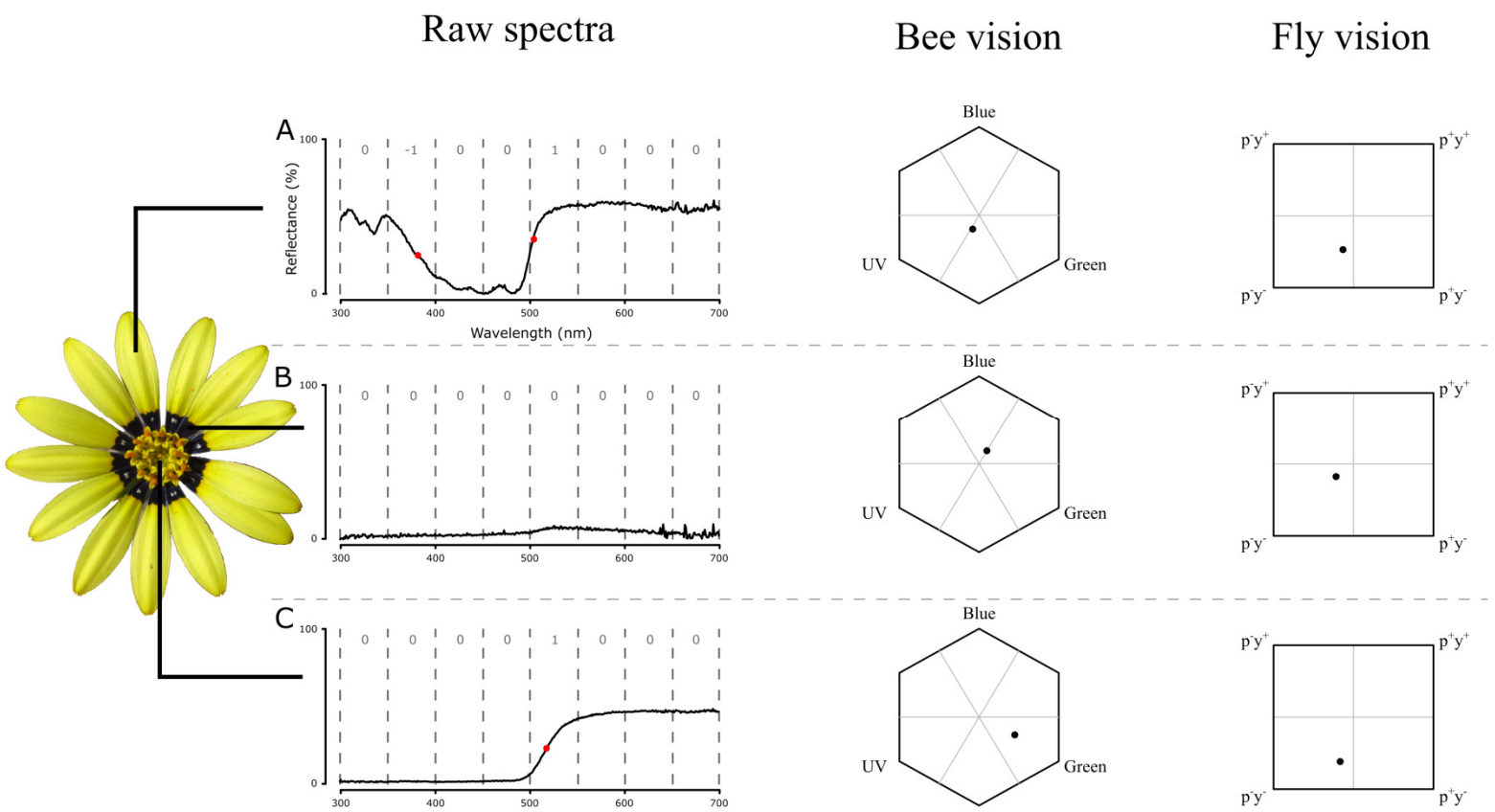

Figure 2.3. Reflectance spectra were measured for three flower sections for each plant species: (A) the outer ray floret (OR); (B) the inner ray floret (IR); and the disc floret (D). To characterise overlapping reflectance curves between species and group similar species into colour pattern

categories (CPCs), inflection points were calculated for raw spectra (indicated as red dots on the curves). For each 50 nm interval, we determined whether an inflection point exceeding a change in reflectance of 10 % was absent (0) or present, and if present, whether it represented a change from concave to convex (-1) or the opposite (1). Each 50 nm interval was then treated as a separate variable in cluster analysis, and cluster analysis was performed for the three flower sections separately. Species with the same combination of OR, IR and D were grouped together in a phenotype. Plant species were also sorted into colour pattern groups based on bee and fly visual models respectively.

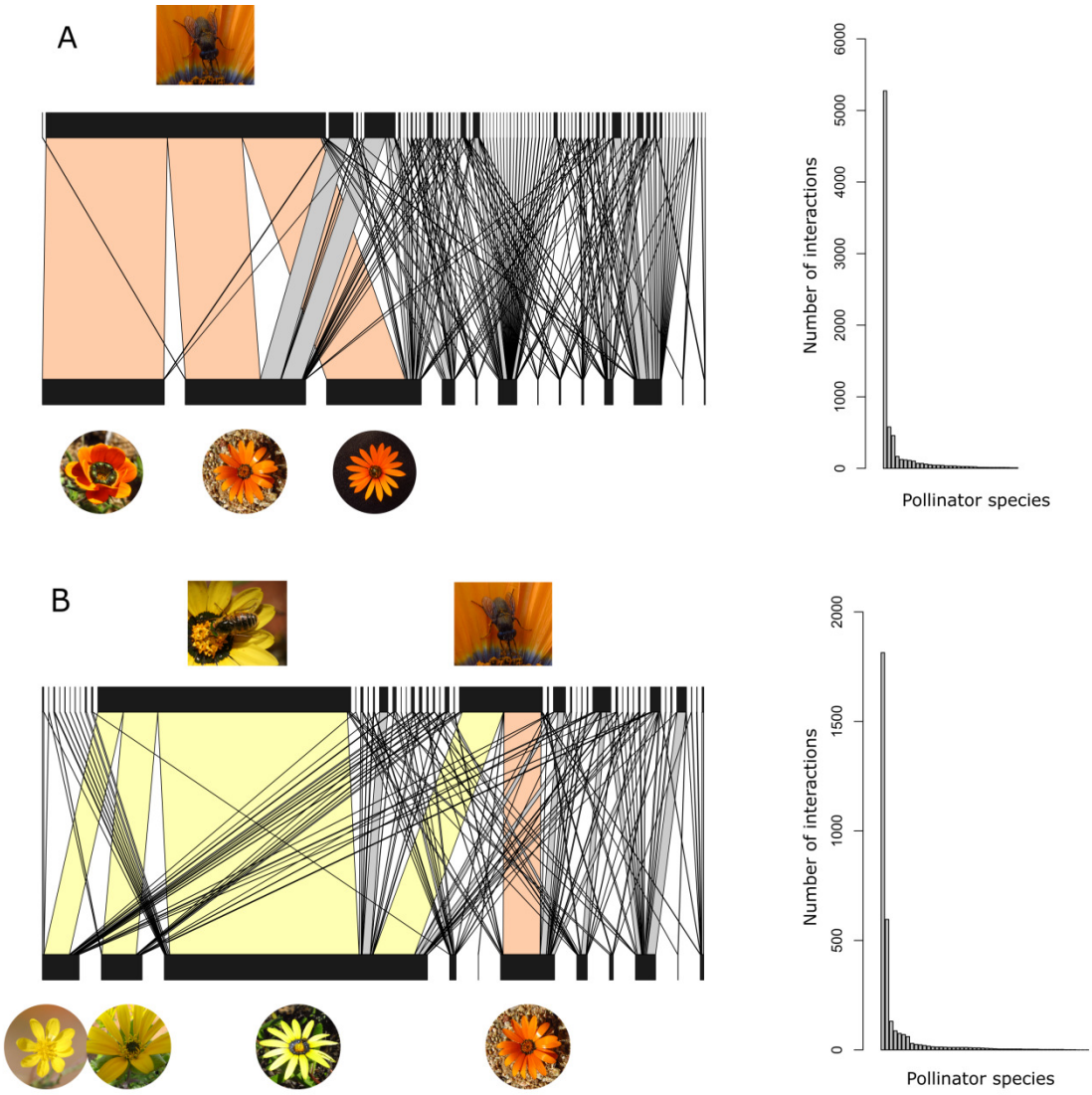

Figure 2.4. Bipartite interaction webs for communities 2 (A) and 7 (B) that exhibited clustered assembly of flower colour. ANOVAs with *a priori* contrasts showed that the dominant pollinators interact significantly more frequently with overrepresented CPCs in both communities. In community 2 (A) a bombyliid fly, *Megapalpus capensis*, was responsible for 68% of observed visits, and in community 7 (B) a tabanid fly, *Rhiogioglossa* sp., is dominant (57% of visits). Graphs on the right indicate interaction frequencies for all pollinator species in these two communities (ranked by interaction frequency) illustrating the

overriding dominance of a single pollinator in each community. Photos by JEK, AGE, NGB, and Bruce Anderson.

Chapter 3

Is the geographic distribution of flower colour in Namaqualand daisies associated with pollinator preferences and distributions?

Jurene E. Kemp, Bruce Anderson & Allan G. Ellis

Abstract

Angiosperms show striking variation in flower colour across and within species. This variation is often attributed to divergent selection by pollinators resulting from geographic variation in pollinator availability and variation between pollinator species in flower colour preferences. Previous work in Namaqualand has shown geographic structure of flower colours, and here we test whether this is associated with pollinator species distributions and divergent pollinator colour preferences. In particular, some Namaqualand communities are dominated by orange flowers and others by white. We make two within-genus colour comparisons to assess whether orange and white flowers are primarily visited by different dominant pollinators. We find that populations of orange flowers are mainly visited by *Megapalpus capensis* and populations of white flowers are mainly visited by *Corsomyza nigripes*. We conduct a series of cage choice experiments to assess whether these two dominant pollinator species could be selecting for alternative floral colours through preference. *M. capensis* has a strong innate preference for orange, which appears not to be learned or influenced by natural soil background contrast. In contrast, *C. nigripes* appears to have an innate preference for white flowers, however detectability is influenced by contrast with natural soil colours. We then quantify the abundances of these two pollinator species across Namaqualand independently from the focal plant species in 100 communities. The two pollinator taxa mostly do not co-occur, and pollinator species densities are associated with which flower colour is dominant across communities. These findings demonstrate a strong geographic mosaic of pollinators with divergent colour preferences that has potentially shaped the landscape-level geographic structure flower colour occurrence in Namaqualand.

Introduction

The vast diversity of flower colour in angiosperms is striking, and closely-related plant species often vary markedly in flower colour or colour patterning (e.g. Cooley et al. 2008; Ellis & Johnson 2009; Newman et al. 2012a; Muchhala et al. 2014; Ellis and Field 2016; Wang et al. 2016). This variation between, and often within species, suggests that flower colour is a labile trait (Wessinger and Rausher 2012; Carlson *et al.* 2015; Koski and Ashman 2015), and that selection frequently acts thereon (Harder and Johnson 2009). Pollinators have long been recognized as important agents of selection on floral traits, and flower colour in particular is a common pollinator attraction trait that can act at both long and short distances (De Jong *et al.* 1992; Podolsky 1992; Hill *et al.* 1997; Horth *et al.* 2014).

Pollinator groups vary in their ability to discriminate between colours (i.e. to identify that two colours are different), and to detect particular colours (i.e. to distinguish an object from its background based on its colour) (Chittka 1992; Troje 1993; Lunau and Knüttel 1995; Hart and Hunt 2007; Avarguès-Weber and Giurfa 2014; Garcia *et al.* 2017). The variation in visual systems between pollinator groups should select for different flower colours when dominant pollinators vary across communities (Rodríguez-Gironés and Santamaría 2004; Newman *et al.* 2012; Shrestha *et al.* 2013; Koski and Ashman 2014; Bergamo *et al.* 2016; Rivest *et al.* 2017). Selection on flower colour might also be dependent on the contrast with the background (Bukovac *et al.* 2017b), and flower colour divergence can potentially occur when flower background colours (such as leaf or soil colour) vary spatially. Thus, pollinators might also favour flower colours that are more detectable in a particular environment, rather than choosing a particular colour *per se*. This may be particularly important for flowers that are presented against soil backgrounds, and in areas where soil colour shows strong spatial

structure. However, the influence of background contrast has mostly been neglected (Bukovac *et al.* 2017b).

If pollinator species exhibit divergent flower colour preferences, and if pollinator species occurrences show geographic structure, then we might expect flower colour to consequently show similar geographic structure due to divergent selection or ecological sorting by pollinators (Stebbins 1970; Thompson 1999). This pollinator-shift model of floral divergence is usually supported by evidence that geographically structured floral ecotypes are visited by different pollinator assemblages (Forest *et al.* 2014; Peter and Johnson 2014; Newman *et al.* 2015; Braunschmid *et al.* 2017). However, differential visitation by pollinators in space could result for several reasons; for instance, abiotic factors can lead to spatial variation in floral traits which subsequently results in spatial variation in pollinator visitation. Alternately, spatial variation in pollinator visitation can result from spatial variation in pollinator occurrences (e.g. Phillips *et al.* 2015). Pollinator-floral ecotype associations thus do not necessarily quantify gradients in pollinator abundances, and pollinator densities need to be estimated independent from focal plant species. If geographic structure of flower colour is driven by pollinators, then we expect to see spatial variation in which pollinator species is dominant and we expect these pollinators to exhibit divergent colour preferences.

Recent work on Namaqualand daisies in South Africa showed that plant species within communities tend to share flower colour patterns, and that differences in colour pattern are often associated with differences in the dominant fly pollinator species (Kemp *et al*., in press). This suggests that some Namaqualand fly species might have different colour preferences, and that geographic variation in dominant pollinator species might result in geographic structure of flower colour patterns. Fly colour preferences can potentially either be innate responses resulting from visual system variation or learned responses associated

with particular rewards, although this remains untested for Namaqualand taxa. The limited work on fly vision has shown four types of central photoreceptors that allows for UV, purple, blue and green to be discerned (Troje 1993). Troje (1993) proposed that flies are tetravariant and see colours in separate categories, rather than in a gradient, but this has only been shown for one fly species (Troje 1993; Lunau 2014b). Different fly species have shown respective colour preferences for yellow (Lunau and Wacht 1994; Sutherland *et al.* 1999; Campbell *et al.* 2010) and violet (Goldblatt 2001, Manning and Goldblatt 1997), whereas others avoid visiting flowers with certain colours (Lunau and Wacht 1994). In Namaqualand, long-proboscid *Prosoeca* flies (Nemestrinidae) are generally associated with pink or purple flowers (personal observation), and choice experiments with the bee fly *Megapalpus capensis* has shown attraction to complex flower ornaments (De Jager and Ellis 2012). Whether Namaqualand fly species show colour preferences and whether these preferences, along with the spatial distribution of the fly species, predict flower colour distribution across the landscape, is not known.

Here we build on previous work which showed that Namaqualand communities are dominated by different flower colours (Kemp *et al*., in press). We focus on two community types, one that is dominated by orange flowers and another that is dominated by white flowers. In both these community types, either (or both) a *Dimorphotheca* or *Ursinia* plant species is abundant, and we focus on an orange and a white species from each of these genera. We first identify which pollinator species dominate the interactions with our focal plant species in each respective community type. Next, we use cage experiments to assess whether the dominant pollinator species from each community type exhibits preferences for orange or white, and we test whether flower detectability varies with background (i.e. soil) colour. We then quantify pollinator occurrence across the landscape independent of plant

species occurrence, and then ask whether pollinator species densities can predict flower colour occurrence across the landscape.

If geographic structure of flower colour is driven by divergent pollinator-mediated selection or by ecological sorting resulting from pollinator preferences, then we expect:

1) that the dominant pollinators exhibit divergent colour preferences,

2) that pollinator species show geographic structure in their occurrence (qualitative or quantitative),

3) that pollinator species distributions are associated with flower colour distributions.

Methods & Materials

Study system

Namaqualand falls within the Succulent Karoo (see Fig. 3.1), which (together with the Cape Floristic Region) forms the Greater Cape Floristic Region (Born *et al.* 2007). Desmet (2007) divided Namaqualand into seven broad bioregions, based on the physical environment, climate and flora. Our study is primarily conducted in two of these bioregions: the Sandveld and the Kamiesberg. The Sandveld consists of marine-derived sands, which can be white, yellow or red in colour. The Kamiesberg consists of granite gneiss dome-shaped hills, with variably grained yellow soils.

In the Sandveld, displays are often dominated by white daisies, and in the Kamiesberg displays are often dominated by orange daisies (data from Kemp *et al.*, in press). Displays in

both these areas often contain high densities of species from *Dimorphotheca* and *Ursinia*, and flower colour is not phylogenetically conserved in Namaqualand daisies (Kemp *et al*., in press). For our study, we focus on one species from each genus that has white flowers (i.e. *D. pluvialis* and *U. speciosa,* Fig. 3.2), and one that has orange flowers (i.e. *D. sinuata* and *U. cakilefolia,* Fig. 3.2). We chose these species as all of them are highly abundant (preliminary data) and each white-orange pair is closely related within each genus. Various character-based approaches have grouped *Ursinia speciosa* and *U. cakilefolia* into the same clade within the genus (Snijman 2013; Magee *et al.* 2014). *Dimorphotheca pluvialis* and *D. sinuata* are very closely related, and the only phenotypic difference between these two species is their flower colour (Snijman 2013), and they can produce hybrids. By using phylogenetically closely related species pairs, we attempt to minimise potential chemical or morphological factors that might differ between species.

Few ecological studies have been conducted on the pollinators of Namaqualand daisies, and work has mostly focused on the bee fly *Megapalpus capensis* (De Jager *et al.* 2011; De Jager and Ellis 2012, 2013; Ellis and Johnson 2010, 2012). Kemp *et al*. (in press) showed that floral visits are dominated by small fly species, but that among others, bees, larger Bombyliid flies and monkeybeetles (Scarabidae) are also present. *M. capensis* has been shown to respond strongly to floral visual cues (De Jager and Ellis 2012), and Kemp *et al.* (in press) showed that different fly species interact with different flower colour patterns across communities.

Are different flower colours visited by different pollinator species?

To assess whether orange and white flowers were visited by different pollinator species in natural conditions, we sampled 27 sites in austral spring 2013 -2015 dominated by either white or orange flowers, where the focal *Dimorphotheca* and *Ursinia* species were abundant.

For each site, we walked multiple transects in a 50 x 50 m area, and recorded the identity and abundances of all insect species present in inflorescences of our four focal plant species. We calculated the number of individuals per flower for each insect species to identify the dominant pollinator species on white and orange flowers respectively, and we will focus on these dominant pollinators for the rest of the chapter.

Do pollinators have divergent flower colour preferences?

To determine whether pollinators preferentially visit particular flowers based on colour, we caught multiple individuals of each of the dominant pollinator taxa at each of the two areas sampled in the previous section (i.e. *Megapalpus capensis* and *Corsomyza nigripes*) and conducted a series of choice experiments. We used flower heads from our four focal plant species in these experiments.

As soil type (and soil colour) varies across the range of our focal plant taxa and background contrast can influence detection abilities of insects (Bukovac *et al.* 2017b), we provided two different soil types as background for the flowers during the choice experiments. One type was red acidic marine-derived soil from the Sandveld, and the other was pale yellow granite gneiss derived soil from the Kamiesberg. Experimental arenas contained the two soil types with 8 flowers (4 orange and 4 white flowers from the same genus) placed 15 cm apart in eppendorf tubes on each soil type (i.e. 16 flowers in total; see Fig. 3.3). Experiments were conducted for both plant genera separately using the same fly individuals. Individual flies of each pollinator species were released separately into the caged arenas, and allowed to sequentially visit flowers. The flower colour as well as soil background which was chosen by a fly, were recorded for each visit. Flies were allowed to make a maximum of 20 choices or

until 5 minutes had passed (median number of choices was 5). We also changed inflorescences after every five trials to avoid nectar depletion.

To test for significant differences in flower colour preference between insect species, we ran a Generalized Estimating Equation (GEE) which allowed us to control for the repeated measures in fly choices. Flower colour choice was set as the response variable, and insect species, soil type, and the interaction between these were set as predictor variables. The model assumed an exchangeable correlation structure where the sequential choices of different fly individuals are equally correlated. A binomial distribution with a logit link function was used to obtain the estimated marginal means and their 95% confidence intervals based on approximate jackknife variance estimates. Models were run separately for the two different plant genera. Fly individuals that did not visit flowers on both soil types were excluded from the data set (*Corsomyza*: 3 of 45 trials were excluded; *Megapalpus*: 6 of 93 trials were excluded). All data were analysed in R (R Core Team 2016) and all GEEs were conducted using the 'geepack' package (Halekoh *et al.* 2006).

To directly assess the influence of background contrast on flower colour choice (i.e. to assess whether background influences detectability), insects were allowed a binary choice in caged arenas between four flowers of *Dimorphotheca* (the same colour as the dominant flower colour in each insect species' native range) on the two soil types (pale yellow versus red). That is, *M. capensis* was allowed to choose between orange *D. sinuata* flowers on two different soil types, and *C. nigripes* was allowed to choose between white *D. pluvialis* flowers. The soil layout was similar to the first experiment (see Fig. 3.3). Only the first choice of each fly was recorded, and inflorescences were replaced after every five trials. To determine whether background (i.e. soil type) influences pollinators' flower choices, chi

square tests expecting no differentiation between soil types were conducted. Analyses were conducted separately for each insect species.

Flower colour preferences can be learned or innate. To distinguish between these, the respective insect species were allowed to feed on an array of non-preferred flower colours of both plant genera. These learning trials persisted for 1 hour per fly. The first choice experiment was then repeated. GEEs were conducted to determine whether a pollinator's choice is altered by conditioning on a different flower colour for an hour. Analyses were run separately for each insect species. The model controlled for repeated measures in insect choice and assumed an exchangeable correlation structure where the sequential choices of different insects are equally correlated. A binomial distribution with a logit link function was used to obtain the estimated marginal means and their 95% confidence intervals based on approximate jackknife variance estimates.

To assess whether 1 hour of learning is sufficient, *M. capensis* individuals were allowed to feed on non-preferred white flowers for one day and were then run through the first choice experiment. Another GEE, with the same structure as those previously described, was conducted to assess whether the *M. capensis* individuals that fed on white flowers for a day showed altered flower colour choices

To what extent do the distribution ranges of these pollinators overlap?

To quantify insect densities across Namaqualand independent of our four focal plant species, we surveyed 100 sites in central Namaqualand (Fig. 3.1) over four years (2013-2016) during austral spring. Some of these sites were the same as those used in the first section, but sampling was expanded to include all daisy species, and not just the four focal daisy species. First, at each site (50 x 50 m) we walked multiple transects and surveyed flowerheads of all

daisy species for *M.capensis* and *C. nigripes* individuals, and from this we calculated the number of insect individuals per flower for each daisy species. We then estimated flower densities in at least twenty $1m^2$ plots sampled on transects across the sites, and from this we could estimate the abundances of both fly species at each site.

To assess whether the two focal pollinator species have overlapping ranges, we used a Fisher's exact test. If ranges are non-overlapping, we expect only one of the two pollinator species to occur at all sites, and we expect them to co-occur at none of the sites. We thus calculated the number of sites where either species occurs and we calculated the number of sites where they co-occur. We tested these observed frequencies against the expected frequencies of non-overlapping ranges (i.e. at 100 sites only one species occurs, at 0 sites they co-occur). If they have overlapping ranges, we expect significant deviation from the expected values.

Are flower colour distributions associated with pollinator species densities?

If pollinator species occurrence predicts flower colour distributions, we expect that the dominant flower colour in a community should be predicted by the local abundances of pollinator species. To test this, we used the data from the previous section in a logistic regression. For the response variable, we coded the dominant flower colour (determined from flower densities) in a binary fashion (i.e. assigning orange as "1" and white as "0"). We then ran two models with the densities of each dominant pollinator species as the respective predictor variables using sites as replicates. We excluded sites from the analyses where none of our focal plant taxa were present (i.e. 43 out 100 were excluded).

Results

Are different flower colours visited by different pollinator species?

Megapalpus capensis was recorded on *D. sinuata* or *U. cakilefolia* (i.e. orange daisies) at 20 out of 22 sites. All other insect species were recorded on these orange flowered species at eight or fewer sites. *Corsomyza nigripes* was recorded at all (i.e. five) sites with *D. pluvialis* or *U. speciosa*, and all other insects were recorded at three or fewer sites. On average, when *M. capensis* occurred on orange flowers, it made up 43% of the insect visitors (on *D. sinuata*: mean = 44%, s.d. = 32%; *U. cakilefolia*: mean = 40%, s.d. = 29%). Similarly, when *C. nigripes* occurred on white flowers, it on average made up 68% of insect visitors (on *D. pluvialis*: mean = 83%, s.d. = 10; *U. speciosa* = mean = 18%, s.d. = 0%). Thus, although other insect species visited orange and white flowers, *Megapalpus capensis* was the most consistent and most frequentvisitor to orange flowers across sites, and *Corsomyza nigripes* was the most consistent and most frequent visitor to white flowers. Further, most of the other insect species observed in flowers were florivorous beetles that did not frequently move between inflorescences.

Do pollinators have divergent flower colour preferences?

For experiments conducted using *Ursinia* plants, 47 *M. capensis* individuals made 482 choices and 20 *C. nigripes* individuals made 131 choices. Fly species exhibited different colour preferences (Wald = 136.56, $p < 0.001$, Fig. 3.4), with *M. capensis* preferring orange and *C. nigripes* preferring white, and this was not influenced by soil type (Wald = 0.16, $p = 0.69$) or the interaction between insect species and soil type (Wald = 1.10, $p = 0.30$). Similar results were found when doing the experiments using *Dimorphotheca* plants, with 26 *M. capensis* making 316 choices and 26 *C. nigripes* making 177 choices. Pollinators showed the same divergent colour preferences as with *Ursinia* (Wald = 119.99, $p < 0.001$), which were

not influenced by soil type (Wald = 0.50, p = 0.48) or the interaction between pollinator species and soil type (Wald = 2.91, p = 0.09).

C. nigripes flies more frequently chose flowers on red marine-derived soils when allowed to choose between white *D. pluvialis* flowers on the two different soil backgrounds (χ^2 = 9.32, p = 0.002, Fig. 3.5). In contrast, *M. capensis* flies showed no preference when allowed to choose between orange *D. sinuata* flowers on different soil types (χ^2 = 0.27, p = 0.60, Fig. 3.5).

Neither pollinator species showed evidence of altered choices after feeding on a non-preferred flower colour for an hour (*Corsomyza*: Wald = 2.96, p = 0.09; *Megapalpus*: Wald: 0.68, p = 0.41, Fig. 3.6). *M. capensis* also did not show a change in preference after feeding on white flowers for a day (Wald = 1.57, p = 0.21).

To what extent do the distribution ranges of these pollinators overlap?

Of the 100 sites sampled, 88 were dominated by *M. capensis* and 12 were dominated by *C. nigripes*. The maximum *M. capensis* density recorded was 1.7 flies per m^2 (average 0.2), and the maximum density recorded for *C. nigripes* was 3.7 flies per m^2 (average 0.8). Fisher's exact test showed that pollinator species have non-overlapping ranges (p = 1), where only one of these two pollinator species occurred at 95 of our sites, and they only co-occurred at 5 sites (Fig. 3.7).

Are flower colour distributions associated with pollinator species densities?

The dominant flower colour across sites was predicted by both *Megapalpus capensis* densities (d.f. = 56, z = 2.68, p = 0.027, and *Corsomyza nigripes* densities (d.f. = 56, z = -2.173, p = 0.03, Fig. 3.8). From the z-scores we can see that high *M. capensis* densities were

associated with the presence of orange daisies (orange was coded as "1"), and high *C. nigripes* densities were associated with white daisies (coded as "0").

Discussion

White and orange daisies were consistently visited by different primary pollinator species. These two dominant pollinator species showed strong flower colour preferences which did not result from short-term learning; that is, *Megapalpus capensis* preferred orange and *Corsomyza nigripes* preferred white. The two pollinator species had largely non-overlapping ranges, which were strongly associated with divergent flower colours. This suggests that pollinator species distributions and pollinator colour preferences jointly influence landscape-level floral trait distributions. We also showed that background (i.e. soil) colour influenced the detectability of flowers for *Corsomyza nigripes*, but not for *Megapalpus capensis*.

Geographic trait matching between plants and pollinators has been shown in many studies (Nilsson 1988; Steiner and Whitehead 1990; Alexandersson and Johnson 2002; Anderson and Johnson 2008; Newman *et al.* 2015). The pollinator-shift model of floral divergence stipulates that geographic variation in floral traits should result from shifts in relative visitation by pollinator species (Grant and Grant 1965; Stebbins 1970). These shifts in relative visitation can result from spatial variation in pollinator availability, variation in pollinator behaviour (e.g. Newman *et al.* 2012), or variation in plant competition for pollinators. Although shifts in pollinator visitation have commonly been shown (Johnson *et al.* 1998; Bradshaw and Schemske 2003; Newman *et al.* 2012; Forest *et al.* 2014; Peter and Johnson 2014), few studies have quantified the reason for the shift. Our work shows that geographic trait matching between plants and pollinators is associated with geographic structure in pollinator occurrences, which suggests that pollinator climates predict the

geographic structure of flower colour occurrence in Namaqualand. Our findings align with Waterman *et al.* (2011) who showed that the geographical structure of dominant pollinator species occurrence in South African orchids results in floral trait clustering that matches pollinator distributions.

Geographic variation in pollinator community composition can drive the divergence or ecological sorting of floral traits. However, pollinator ranges are rarely quantified (but see (Phillips *et al.* 2015). Here we show that two dominant pollinator species in Namaqualand have non-overlapping ranges, which suggests that the two fly genera have different abiotic or biotic requirements which restrict them to certain areas, and this potentially indirectly limits plant species distributions (Pellissier *et al.* 2012). Bombyliidae generally have larval forms that parasitize nearly any insect species (Yeates and Greathead 1997), but nothing is known of the hosts of *Megapalpus* or *Corsomyza*, or of their abiotic requirements. As *M. capensis* readily fed on white flowers when orange flowers were unavailable in the learning experiments, it is unlikely that flower colour is limiting the distribution range of *M. capensis*.

Flower colour evolution is influenced both by pollinators' ability to detect flowers (i.e. to distinguish the flower from its background) and to discriminate between flower colours (i.e. to distinguish between flowers with different reflectance spectra) (Shrestha *et al.* 2013; Bukovac *et al.* 2017a). We showed that both *M. capensis* and *C. nigripes* can discriminate between white and orange colours. Recently, Bukovac *et al*. (2017b) used visual space modelling to show that flower colours viewed against different background colours can influence what colour is perceived in bee vision, and it can influence whether the flower can be distinguished from the background. Interestingly, background contrast influenced *Corsomyza nigripes* choices, but it did not override flower colour preference, which suggests that background contrast influenced the detectability of white flowers for *C. nigripes*. In

contrast, *M. capensis* preferred orange flowers, irrespective of background colour. This potentially suggests that *M. capensis* can distinguish well between colours in the long wavelength range (i.e. between orange and red), or it uses other cues to detect flowers. Experimental work that tests the visual capabilities and colour preferences in fly species remains scarce (but see Lunau and Wacht 1994; Dinkel and Lunau 2001; Shrestha *et al.* 2016; Lunau *et al.* 2018), despite mounting evidence that flies are important pollinators (Orford *et al.* 2015). In addition to the lack of information on visual system functioning and variance therein between species, we also know very little about the foraging behaviour of fly pollinators. *Megapalpus capensis* does not exhibit constancy between orange daisies that vary in ornamentation (i.e. rings or mate-mimicking spots - Ellis and Johnson 2012), which contrasts the behaviour of bees (Hill *et al.* 1997). The rings and spots commonly found on flowers visited by *M. capensis* potentially increase visitation rates (De Jager and Ellis 2012), but not floral constancy, and the increase in visitation rates might be enough to increase the probability of pollen being transferred between conspecifics.

Determining the extent to which colour preferences are learnt or innate is often a difficult task. Some pollinator species, particularly bees, show short term learning, that is, they learn to associate a particular floral trait with rewards and then sequentially visit flowers with that trait for a short time period (Chittka *et al.* 1999, Dyer & Chittka 2004). The bee flies in our experiments showed no signs of short term learning, and *M. capensis* showed no indication of learning after feeding on the non-preferred flowers for a day. Unfortunately, the *C. nigripes* flies did not survive on non-preferred flowers for a day, and all specimens died. This may either indicate that flies could not learn to use the novel resource or they were not able to use it due to chemical or morphological mismatches, and this requires further investigation. Interestingly, after spending an hour on orange flowers, *C. nigripes* flies chose nearly significantly more ($p = 0.09$) white flowers than before forced feeding, which suggests that

they may have learned to avoid orange flowers. Previous work has shown that *M. capensis* can learn that mate-mimicking spots are deceptive, and consequently they reduce their mating response to these spots, which suggests that *M. capensis* can learn to avoid non-rewarding resources (de Jager and Ellis 2014).

Our results align with previous work which suggested that pollinator preferences might be influencing Namaqualand floral trait assembly through facilitation, filtering or convergence (Kemp *et al*., in press), and here we provide evidence for community level convergence and filtering of flower colour resulting from pollinator preferences. In addition, particularly for the closely related *Dimorphotheca* species pair, flower colour divergence may have occurred across the strong geographic mosaic of pollinators with divergent colour preferences that we demonstrate. Other species in this region, such as *Dimorphotheca pinnata* and *Gorteria diffusa* (Ellis and Johnson 2009), also show flower colour polymorphisms (Kemp et al, in press), suggesting that flower colour adaptation to locally dominant pollinators might be prevalent in this region. The strong colour preferences we demonstrate could also act as a biotic filter that prevents plant species with non-preferred flower colours from infiltrating communities (Sargent and Ackerly 2008), thus limiting plant species distributions. Our findings align with those of Shrestha *et al.* (2016) who showed that fly pollinators act as a biotic filter of plant immigrants to Macquarie Island, where other pollinator groups are absent due to harsh abiotic conditions, and only plants with cream-green flowers can persist there.

Conclusion

We show closely-related bee fly species prefer different flower colours, and this does not appear to be a learnt association. These fly species have non-overlapping ranges, suggesting they have different resource requirements, which might be related to their parasitic larval

stages. Pollinator abundances, quantified independently of the focal plants, predict the flower colour of *Dimorphotheca* and *Ursinia* daisy species.

Figures

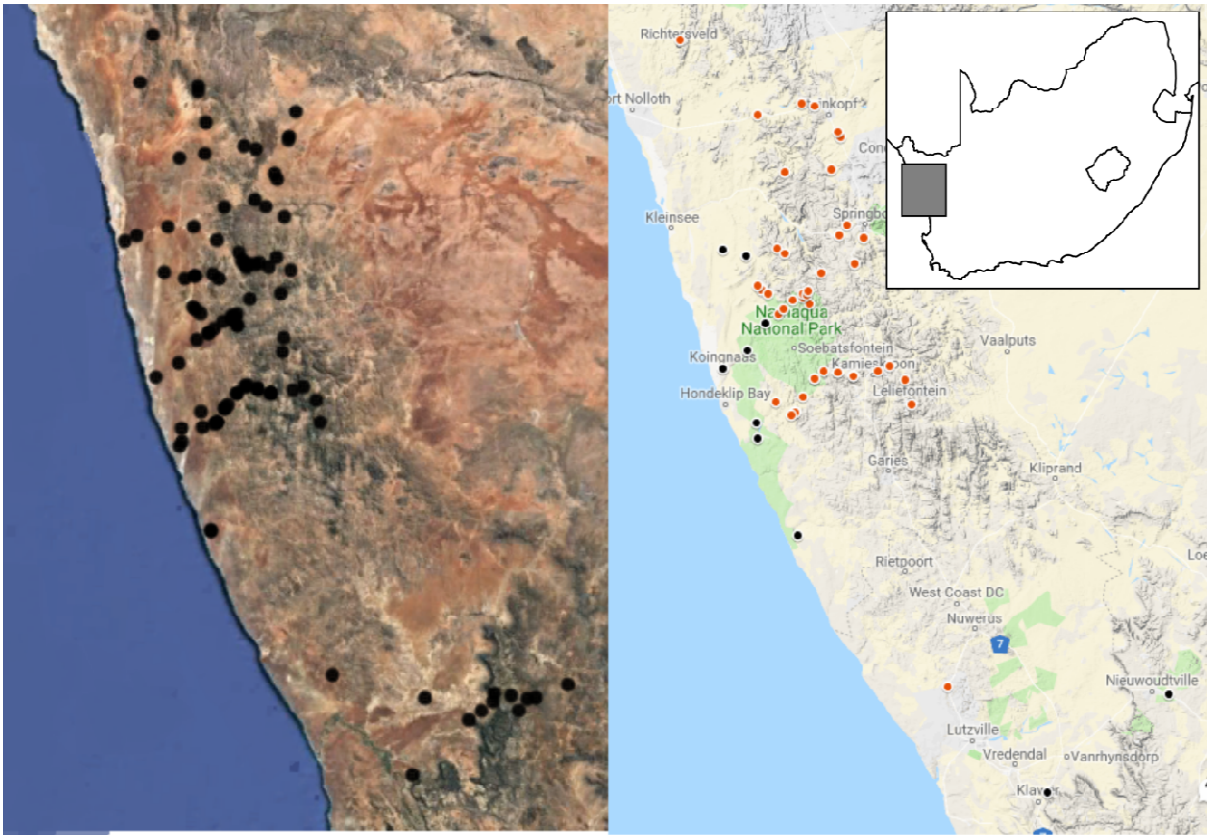

Figure 3.1. Map of 100 sites sampled for insect densities (indicated with black dots) is shown on the left. The map on the right shows the 57 communities where our four focal plant species occurred, and communities dominated by orange flowers are indicated with orange dots and communities dominated by white flowers are indicated with black dots.The inset shows in grey where Namaqualand is located within South Africa

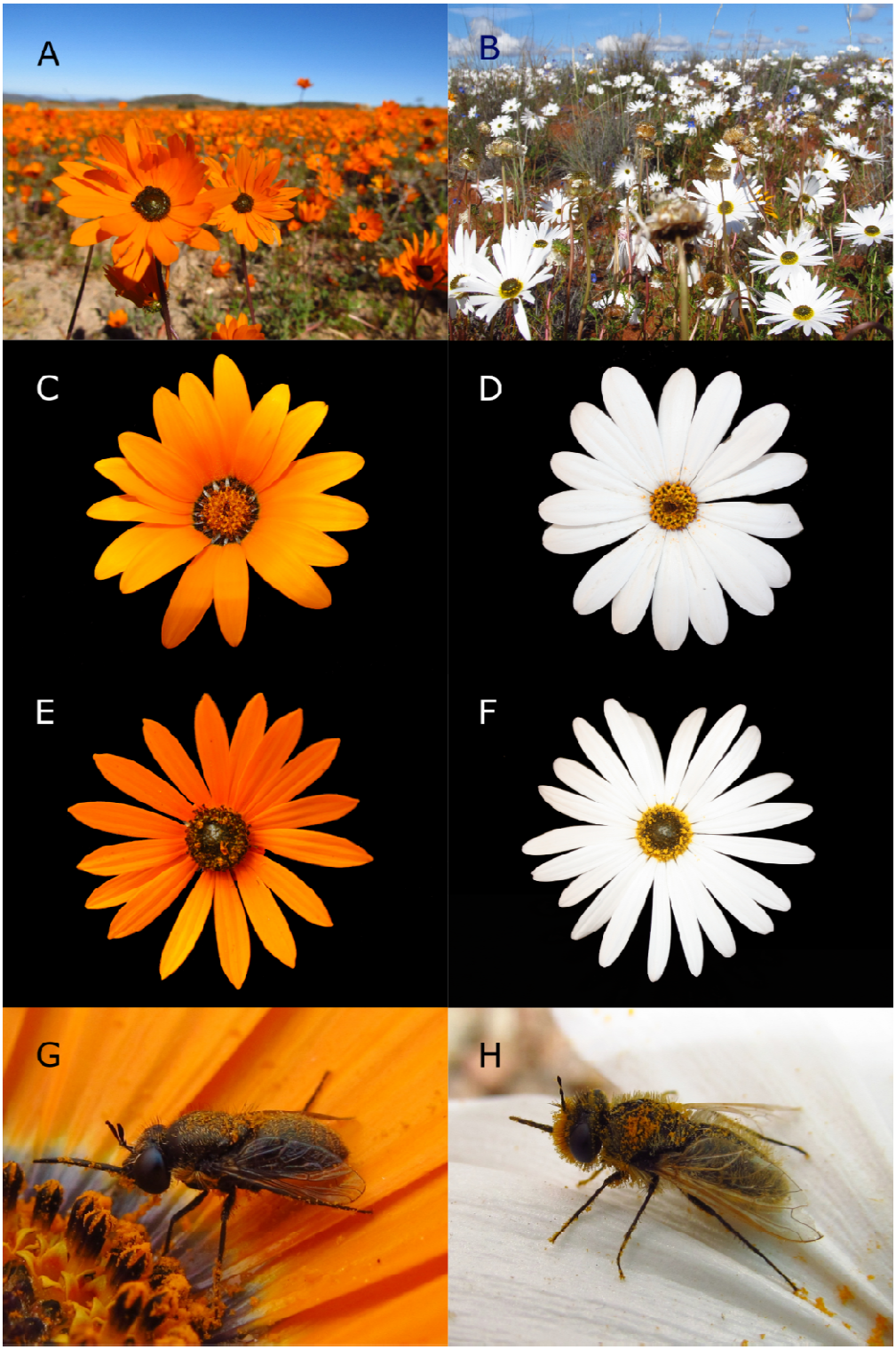

Figure 3.2. The plant species and their pollinators that characterize white and orange dominated communities in Namaqualand. (A) A Namaqualand community dominated by

orange flowers. (B) A Namaqualand community dominated by white flowers. (C) *Dimorphotheca sinuata*, a common daisy in Namaqualand. (D) *D. pluvialis*. (E) *Ursinia cakilefolia*. (F) *U. speciosa*. (G) *Megapalpus capensis*. (H) *Corsomyza nigripes*, covered in pollen.The inflorescence diameter of the two *Dimorphotheca* species is on average 35 mm, and the diameter of the two *Ursinia* species is on average 31 mm. Both fly species show variation in size, but are always smaller than 10 mm.

Figure 3.3. The experimental setup for flower colour choice experiments is shown. (A) Eight white and eight orange flowers were placed on two soil types (red marine-derived soils – B; and pale granite gneiss-derived soils – C). *Megapalpus capensis* and *Corsomyza nigripes* bee fly individuals were released into caged arenas containing the depicted setup, and both flower colour and background choices were recorded. The experiments were first conducted using an orange-white pair of *Dimorphotheca* flowers, and then a pair of *Ursinia* flowers.

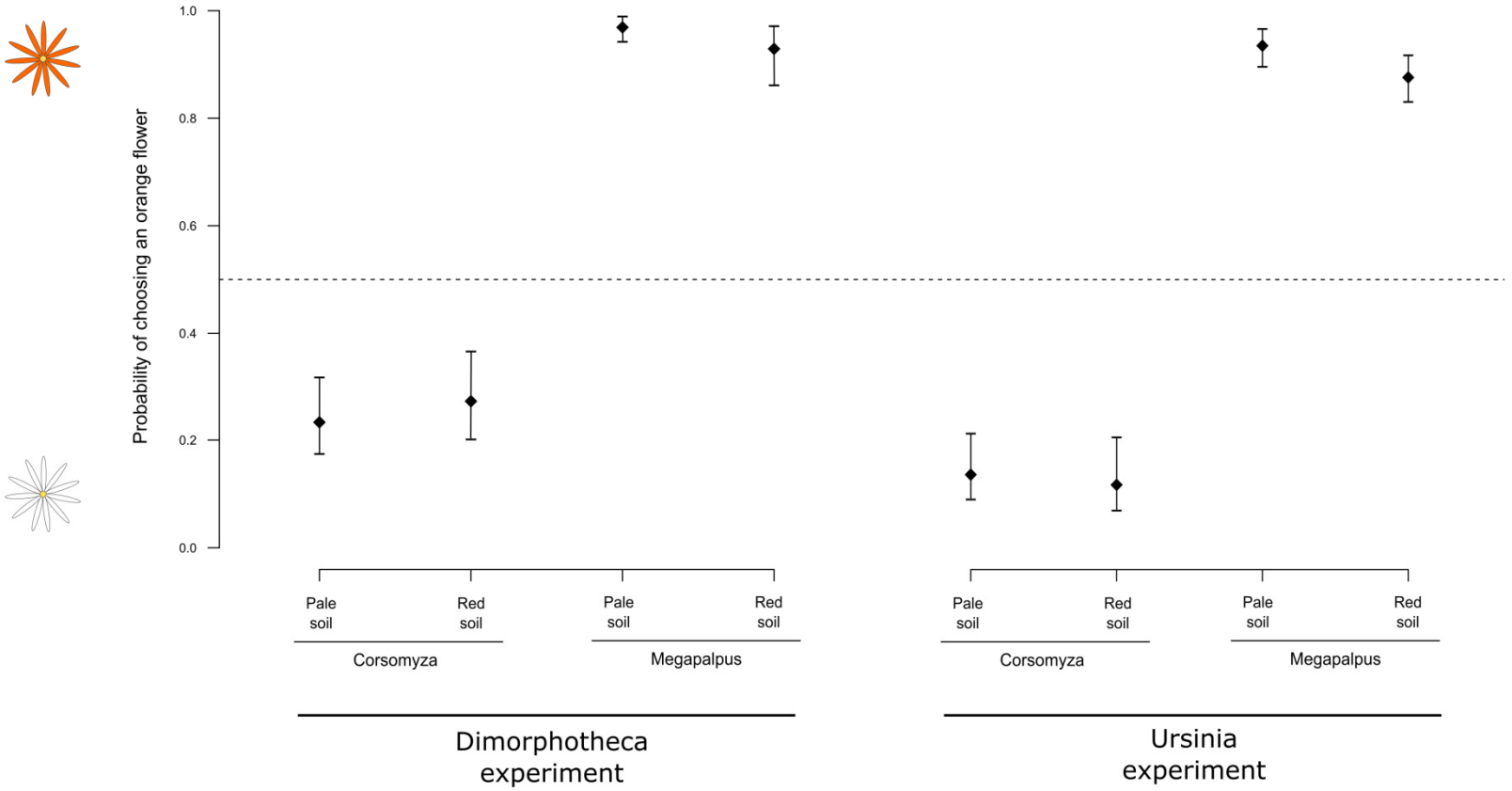

Figure 3.4. The probability of pollinating flies (*Corsomyza nigripes* and *Megapalpus capensis)* choosing an orange flower over a white flower. The first experiment tested for fly colour preferences within the genus *Dimorphotheca* (orange *D. sinuata* versus white *D. pluvalis*) while the second experiment tested for preferences within the genus *Ursinia* (orange *U. cakilefolia* versus white *U. speciosa*). In each experiment flower

choices were made on both red and pale soils. *M. capensis* shows a preference for orange flowers, regardless of the colour background, and *Corsomyza nigripes* shows a preference for white flowers, irrespective of the background colour.

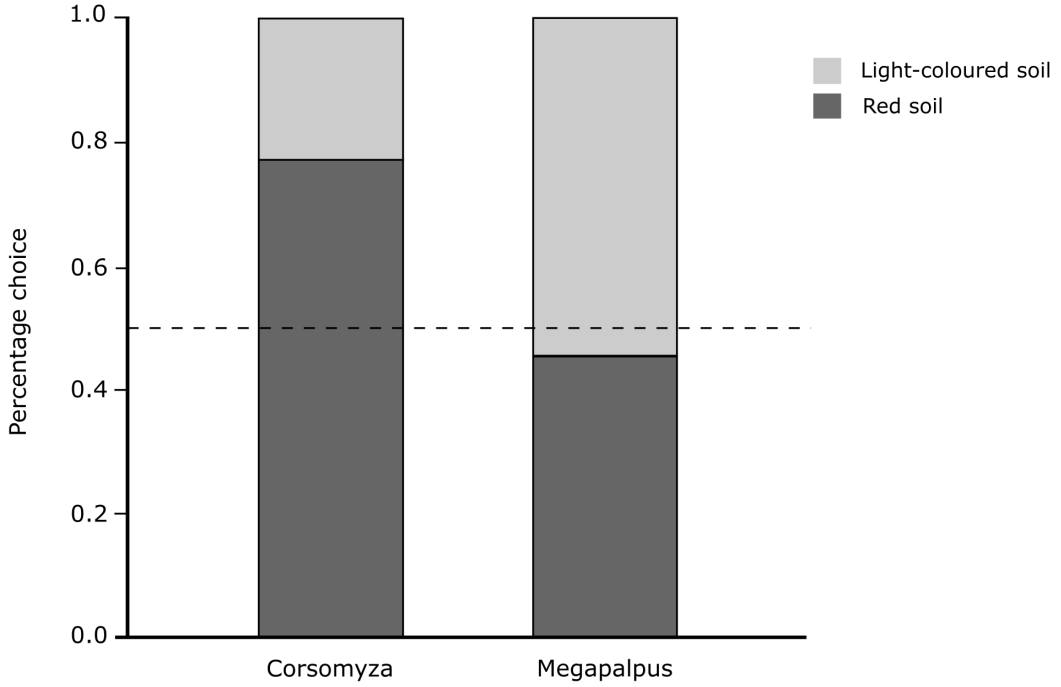

Figure 3.5. The effect of floral colour and soil colour contrasts on pollinator foraging behaviour. *Corsomyza nigripes* (usually found in white floral communities on red soils) was offered a choice between white flowers (*Dimorphotheca pluvialis*) on pale versus on red soil. *Megapalpus capensis* (usually found in orange floral communities on pale soils) was offered a choices between orange flowers (*D. sinuata*) on pale versus red soil. *C. nigripes* visited flowers disproportionately more on red soils, and *M. capensis* showed no preference for either background contrast. This suggests *C. nigripes* detects white flowers less well on light coloured soils than on red soils, but *M. capensis* shows no difference in detection ability. The dashed line indicates the expected choice ratio if there is no difference in detectability.

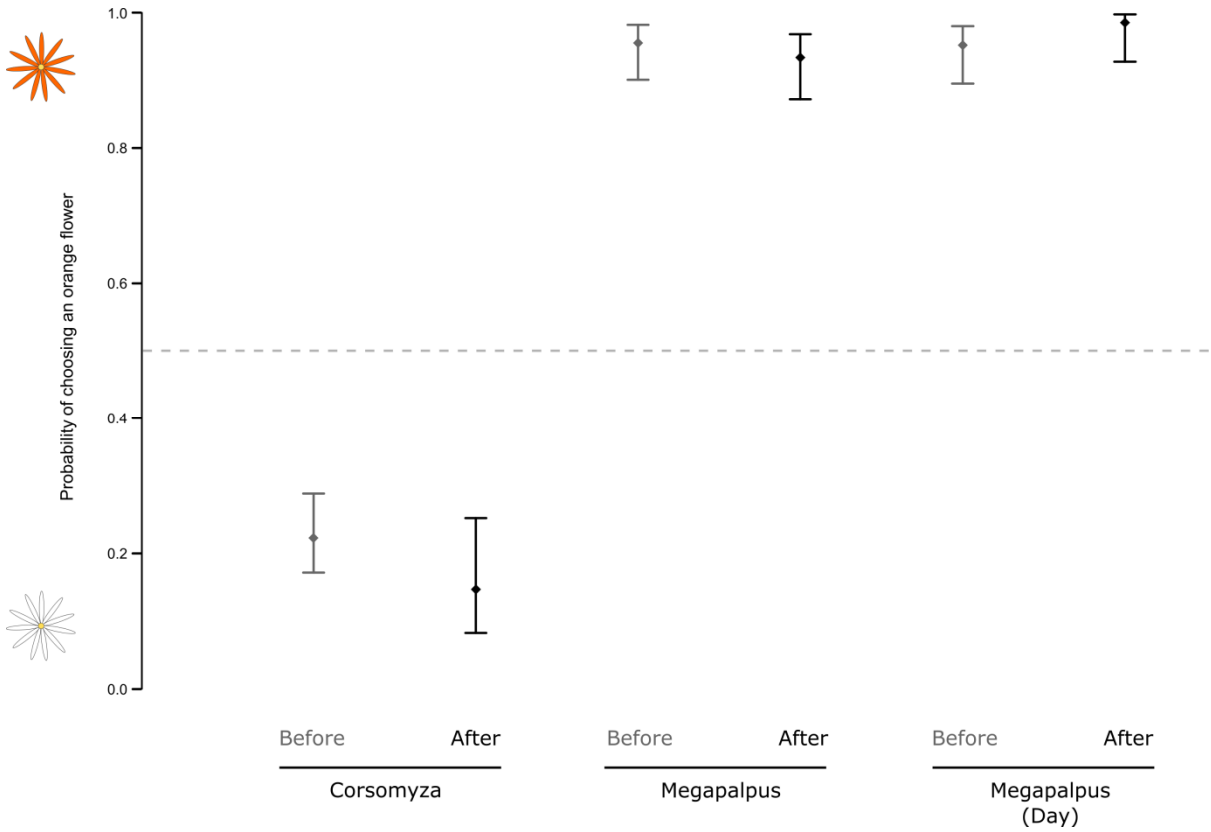

Figure 3.6. The effect of colour conditioning on the flower colour choices of two dominant fly pollinator species. *Corsomyza nigripes* and *Megapalpus capensis* flies were forced to feed on flowers of their non-preferred colour for one hour, and additional *M. capensis* flies were forced to feed on white non-preferred flowers for a day. We repeated flower colour choice experiments with the same flies after conditioning. Here we show the choice probabilities before and after conditioning. We saw no effects of either *C. nigripes* or *M. capensis* flies learning to utilize the non-preferred resource.

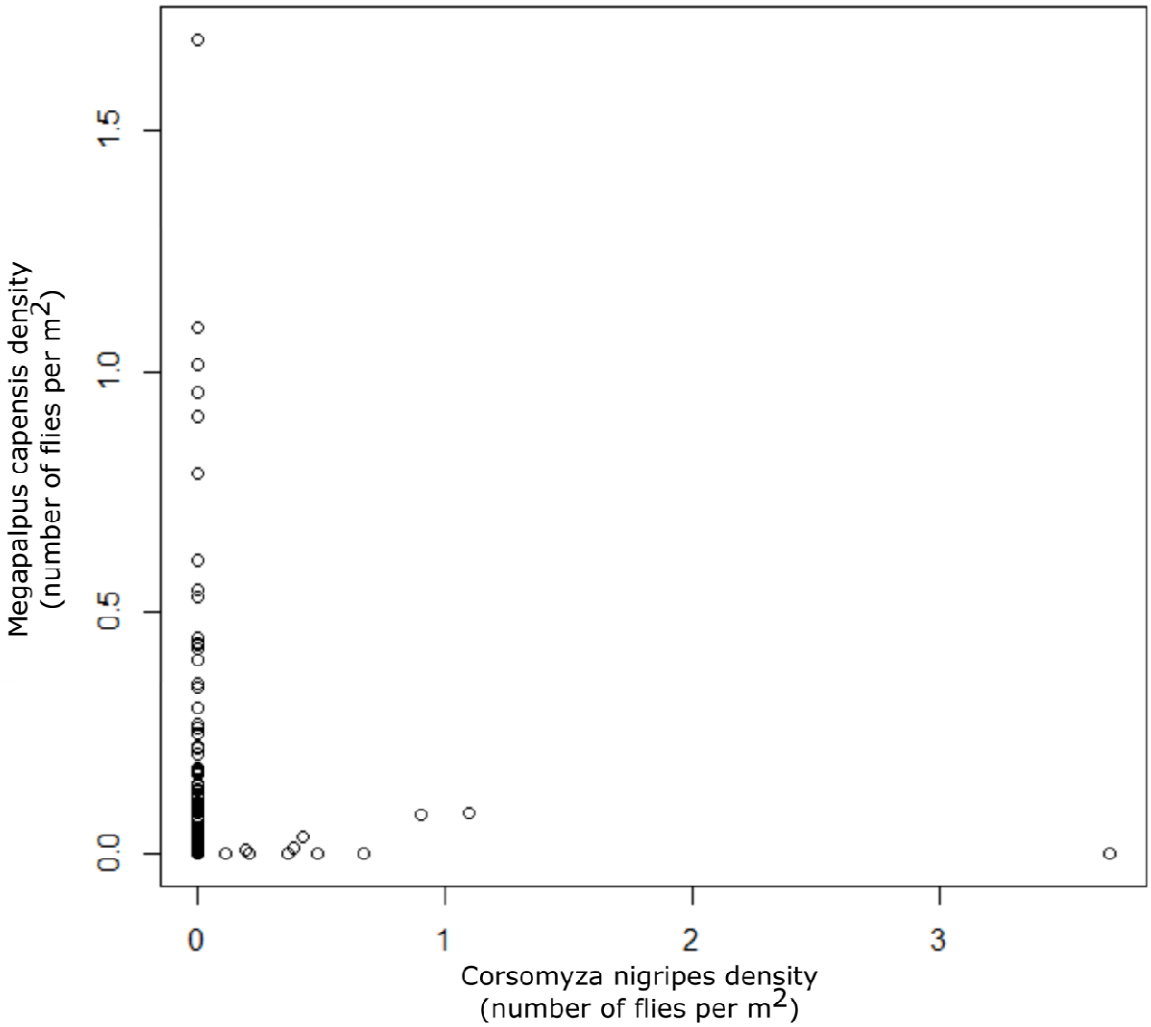

Figure 3.7. The densities of *Megapalpus capensis* and *Corsomyza nigripes* were quantified independent from focal plant species across 100 sites. The densities of both species at each site are plotted against one another, and shows that the two bee fly species have largely non-overlapping ranges.

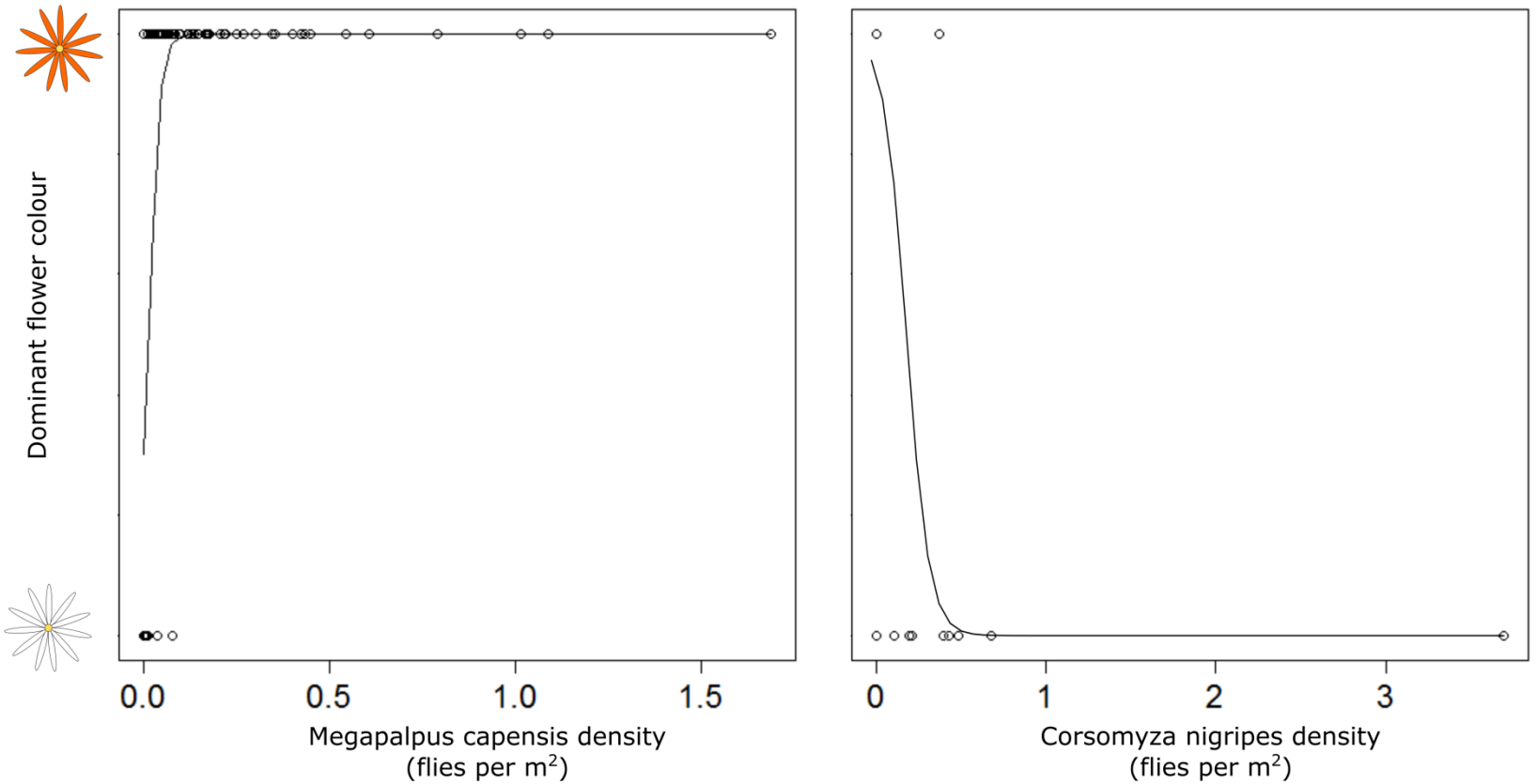

Figure 3.8. The association between dominant flower colour and pollinator species densities is shown. *Megapalpus capensis* and *Corsomyza*

nigripes densities predict flower colour dominance across 57 sites, where *M. capensis* is associated with orange flower dominance ($z = 2.680$, $p = 0.007$) and *C. nigripes* is associated with white flower dominance ($z = -2.173$, $p = 0.03$).

Chapter 4

Cryptic petal colouration decreases floral apparency and herbivory in nocturnally closing daisies

Jurene E. Kemp & Allan G. Ellis

Abstract

It is increasingly recognised that floral traits are shaped by diverse selection agents, including pollinators and antagonists. Floral apparency, for example, increases pollinator visitation, but this benefit likely trades-off against the potentially severe fitness costs of damage to, or removal of, apparent flowers by floral herbivores. Previous work has suggested that closing flowers at night decreases herbivory rates by decreasing visual signal size to herbivores when pollinators are inactive. Here we test whether closing species have evolved less visible lower petal surfaces that are inconspicuous to herbivores when flowers are closed. We collected lower and upper petal surface spectra for 77 Asteraceae species that flower during the annual Namaqualand mass display, and modelled these in herbivore vision. Closing species had larger differences between upper and lower petal surface colouration than non-closing species, and closing species' lower surfaces were less visible to herbivores than those of non-closing species. We confirmed a fitness advantage of cryptic flower colour with controlled herbivory experiments, which showed higher herbivory rates for open flowers than closed flowers that result from a difference in colouration of the visible petal surface. Visual crypsis of flowers may thus be an effective anti-herbivory defence strategy during times when pollinators are inactive, and provides an alternative to chemical defence which often has costs to pollination success.

Introduction

As flowers are primarily signals for pollinator attraction, pollinators should select for floral traits, such as visual or olfactory cues, that increase floral apparency and attractiveness (Fenster *et al.* 2004; Sargent and Ackerly 2008). However, increased conspicuousness of flowers comes at the cost of attracting antagonists (McCall and Irwin 2006). Floral traits thus ultimately represent trade-offs between selection to attract pollinators and selection to reduce antagonistic interactions, and this trade-off is increasingly being recognised as an important determinant of floral trait evolution.

Plant fitness can directly be reduced by insect florivores that damage or consume pollen and ovules, whereas larger herbivorous animals reduce fitness by consuming entire flowers (McCall and Irwin 2006). Like many pollinators, specialist florivores and herbivores use flowers as an important source of nutrients, particularly of protein. Florivory can also have indirect effects on plant fitness by altering pollinator regimes (Gómez 2003; Strauss and Whittall 2006; Sõber *et al.* 2010). Florivore damage alters aspects of the floral display, such as flower shape or size (Zangerl and Berenbaum 2009; Cardel and Koptur 2010), which in turn alters pollinator foraging behaviour.

Chemical defence of vegetative tissue commonly protects plants against herbivores, and the same compounds involved in defending vegetative tissue are often present in floral tissue (Adler 2001; Strauss *et al.* 2004; Irwin and Adler 2006). However, plant species that are reliant on pollinators exhibit lower levels of defence compounds in flowers, which suggests a trade-off between chemical defence and pollination (Adler *et al.* 2012). A possible way to circumvent this trade-off, particularly relevant in the context of floral apparency, is that plants can potentially

avoid herbivory through visual crypsis. Although crypsis is widely reported in animals as an anti-predation strategy, it is not frequently reported in plants (but see Strauss & Cacho 2013, Niu *et al.* in press). While visual crypsis in flowers as anti-herbivory defence may seem counterintuitive, visual cues are not always the primary mechanism plants use to attract pollinators (Johnson 1995). Many plants attract pollinators primarily through olfactory cues which potentially allows plants to decrease their visual apparency to avoid visually cueing florivores. However, the absence of strong visual cues in the presence of alternate attraction cues is not usually considered as a mechanism to reduce herbivory (Johnson *et al.* 2007; Shuttleworth and Johnson 2010).

Another way to reduce floral apparency to herbivores, which we consider here, is to only open flowers when pollinators are active, and decrease flower visibility at other times. Repeated diurnal petal movements have been widely recorded (Stirton 1983), and the nocturnal closure of flowers has been recorded in plant species that are pollinated during the day. While the genetic and mechanical aspects of repeated floral closure have been widely explored (reviewed in Van Doorn and Van Meeteren 2003; Van Doorn and Kamdee 2014), the function and evolutionary drivers of floral closure have received less attention. Closure of flowers at night and during inclement weather has been shown to protect pollen from moisture damage (von Hase *et al.* 2006) and to promote ovule fertilization (Liu *et al.* 2017). Regardless of the primary function for flowers closing at night, it may provide an additional advantage of decreased apparency to herbivores (Prokop and Fedor 2016). Recently, Prokop & Fedor (2016) showed that flowers which remain open at night suffer higher predation rates by mammalian herbivores than those that close. They suggest that this anti-herbivory benefit arises due to the smaller display size of closed flowers, thus reducing their apparency to visually cueing herbivores. Further, it has

anecdotally also been reported that lower (abaxial) petal surfaces that are exposed in closed flowers are cryptically coloured, which may lead to flowers being inconspicuous when pollinators are not active (Archibald *et al.* 2004; von Hase *et al.* 2006).

Nocturnal flower closure is widespread in the Greater Cape Floristic Region (GCFR), South Africa, and particularly common during the spring mass flowering displays that are a feature of the Namaqualand subregion (von Hase *et al.* 2006). Diurnal flower opening in Asteraceae that dominate these displays is cued by rising temperatures (von Hase *et al.* 2006), resulting in capitula only being open for a few hours on warm days. Our observations and some reports suggest that mammalian and reptile (tortoise) herbivores feed extensively on flowers during the flowering period, and that many daisies that close nocturnally seem to have cryptically coloured lower petal surfaces. One possibility is that while upper (adaxial) petal surfaces are under selection to increase apparency to pollinators, lower (abaxial) surfaces of closing species may be under selection to reduce apparency to flower feeding herbivores. Here we use closing and non-closing Asteraceae species that flower during the spring mass display in Namaqualand to test four predictions derived from the hypothesis that lower petal surfaces in closing daisies are cryptic and reduce herbivory.

1) Closing species should have larger differences in apparency between upper and lower petal surfaces than non-closing species.

2) Because apparency differences between petal surfaces could arise from selection by pollinators for bright upper surfaces, we also tested for colour (chroma) differences between petal surfaces of closing species which would be expected if the visual properties of upper and

lower petal surfaces are moulded by different selective agents with different colour vision systems (i.e. by pollinators and floral herbivores respectively).

(3) Lower surfaces of closing species should be less visible to potential herbivores than those of non-closing species.

(4) Cryptic colouration of lower petal surfaces in closing species should result in reduced herbivory rates compared to non-closing species or non-cryptic closing species.

Methods

Study system

The semi-desert Namaqualand region of South Africa forms part of the Succulent Karoo, a biodiversity hotspot which, along with the Cape Floristic Region, forms part of the Greater Cape Floristic Region (GCFR). Diurnal petal movement is estimated to occur in approximately 3500 South African plant species (von Hase *et al.* 2006). Namaqualand contains more than 400 Asteraceae species in ~55,000 km^2, making the Asteraceae the most diverse family in this region. Namaqualand receives winter rainfall which results in a short period of mass flowering of predominantly annual plant species. Most of these species are self-incompatible (De Waal *et al.* 2014) and reliant on successful seed set (dependent on pollination and florivory rates) in order to persist across years. Individual daisy capitula last for multiple days, often up to a week (Kemp unpublished data), which increases the probability of an individual flowerheads being eaten. Nocturnal closing in Asteraceae is conserved at the genus-level (Stirton 1983), where ray florets

of some genera recurve (i.e. fold backwards), some remain open, and others close upwards. We treat the latter category as "closing" flowers, as their lower petal surfaces are exposed in the closed state, and their upper surface in the open state. Flowers with recurved petal closure and flowers that do not close present only their upper petal surfaces in the open and closed states, and both of these we thus treat as "non-closing". The opening of daisies in Namaqualand is primarily associated with ambient temperature (von Hase *et al.* 2006), and flowers usually open mid-morning (around 10h00) and close late in the afternoon (around 15-16h00). As sunrise is usually before 07h00 and sunset around 19h00, the lower petal surfaces of closed capitula are thus exposed for many hours during the day, as well as at night. Various studies have shown that the visual phenotype of upper petal surfaces of Asteraceae species in this region is under selection from pollinators (Ellis and Johnson 2010; De Jager and Ellis 2012, 2013, 2014).

Colour measurement

Upper and lower ray floret surface reflectance data between 300 and 700 nm were collected for 77Asteraceae species in the GCFR during austral spring in 2007, 2015 and 2017 (Fig. 4.1). For 71 out of the 77 species we sampled, we recorded their closing state. Spectra were measured indoors at a 45° angle using an OceanOptics USB4000 Spectrometer (accuracy: 0.37 nm; calibration: diffuse reflectance WS-2 white standard). Three to five samples were taken per species and averaged. Spectral curves were smoothed using LOESS smoothing with smoothing parameter of 0.1, binned into 1 nm bins, and negative values were converted to zero. For all spectral data processing and visual space modelling, we used the 'pavo' package (Maia *et al.* 2016) in R (R Core Team 2016).

Colour vision modelling

We modelled upper and lower petal surfaces using the Receptor-Noise Limited (RNL) model (Vorobyev and Osorio 1998) which makes general assumptions about how spectra are processed and can thus be applied across species with different visual systems. The RNL model assumes that chromatic and achromatic contrasts are processed independently, and here it calculates whether an object can be distinguished from a green leaf background. Colour discrimination thresholds (just noticeable differences – JNDs) produced by the RNL model are based on both receptor noise (defined by Weber's law) and neural processing abilities. JND values larger than one show that an animal can distinguish between colours.

Abaxial and adaxial surfaces were modelled in chameleon (*Bradypodion pumilum*) and ungulate (both goat and horse) visual space to assess whether these surfaces vary in visibility to herbivores. Chameleon visual space (Stuart-Fox *et al.* 2007) was used since tortoise visual models are not available, and chameleons represent a reptile taxon with characterised visual system. Sensitivity peaks for chameleon vision are at 365 nm, 443 nm, 483 nm, and 571 nm. We used a pigment density ratio of 1:1:3.5:6 for chameleon vision, as previously used for other reptiles (Barbour *et al.* 2002). For achromatic vision, chameleons use a double cone with a sensitivity peak at 619 nm. As Weber fractions for chameleons are unknown, we used a value of 0.1 (following Fleishman *et al.* 2016). Ungulate vision space was based on the pigments of goats (peaks: 443 nm & 553 nm - Jacobs et al. 1998) and horses (428, and 539 nm - Carroll et al. 2001) respectively, and we used pigment density ratios of 1:1. For achromatic vision, the long wavelength pigment was used for each respective ungulate (Jacobs 1993, Osorio & Vorobyev 2005). A Weber fraction of 0.45 was used for both goats and horses, as this has been shown to be

the Weber fraction for horses (Geisbauer *et al.* 2004). Standard green leaf background and standard D65 daylight illuminance was used in all models.

Dichromats, such as ungulates, tend to use chromatic vision during the day and switch to achromatic vision when light quality decreases, and this has been shown to apply to horses (Roth *et al.* 2008). However, Weber fractions (on which receptor noise depends) become higher at suboptimal lighting conditions and depend on the amount of photons captured during an integration time period (see Olsson *et al.* 2017). Due to the absence of information on how Weber fractions should be adjusted under low light conditions for our focal animal species, we calculate achromatic contrasts with unadjusted Weber fractions, which thus assumes higher achromatic discrimination abilities than actual abilities. However, as we are directly comparing plant taxa subjected to the same modelling approach, the relative differences between petal surfaces within and between species should be accurate. Also, these daisies are often closed for up to five or six hours during daylight hours, which makes chromatic contrasts relevant.

Are lower petal surfaces of closing daisies less apparent to herbivores than upper petal surfaces?

Selection by pollinators is expected to act on upper petal surfaces of both closing and non-closing species, whereas selection on lower petal surfaces by herbivores is only expected in closing daisies. If lower petal surfaces have been selected to be cryptic, we thus expect a larger difference in visibility between upper and lower petal surfaces in closing daisies than in non-closing daisies.

To test this, we calculated the difference in JND values between upper and lower surfaces for each plant species, and tested whether this difference varies between closing and non-closing species. If lower surfaces are adapted to be less visible than upper surfaces, we expect ΔJND (i.e. $JND_{upper} - JND_{lower}$) to be lower in non-closing daisies than in closing daisies. We test for differences in ΔJND between closing and non-closing daisies using a nonparametric Wilcoxon rank sum test for all measured JND values (i.e. chromatic: chameleon, goat, horse; achromatic: chameleon, goat, horse). Because closing is conserved at the genus-level, we also conducted phylogenetically corrected ANOVAs for these comparisons using the 'geiger' package in R. For this, we used the genus-level phylogeny constructed by Kemp *et al.* (in press).

We further tested whether lower surfaces of closing daisies are less visible to herbivores than those of non-closing daisies, as expected if selection by herbivores reduces visibility of lower petal surfaces in closing species. We used one-tailed Wilcoxon rank sum tests to test whether lower surface JND values are lower in closing daisies than non-closing daisies for all herbivore vision models (achromatic and chromatic).

Do closing daisies produce different colours on their upper and lower petal surfaces?

If selection by herbivores is acting on the apparency of the lower petal surface in closing daisies, i.e. if different selective agents with different visual systems are selecting on the two respective petal surfaces, we might expect chroma differences between upper and lower petal surfaces for closing daisies, whereas in non-closing species we might rather expect lower intensity of the same colour pigments in lower petal surfaces compared to upper petal surfaces.

To assess this, we first quantified flower chroma (irrespective of intensity) for each plant species. We did this by calculating inflection points on the spectral reflectance curve (i.e. points where the curvature of the reflectance spectra change), which explicitly quantifies chroma. We used the method of Kemp *et al* (in press) and divided the spectral curve into eight 50 nm bins, and recorded when an inflection point occurs in a particular bin. Changes from concave upward to concave downward were scored as 1, and the reverse was scored as -1. We then used the eight bins in a principal component analysis (PCA) to reduce the number of variables. Chroma that are more similar to each other should be closer together in the PCA space. Subsequently, we calculated Euclidean distances between points on the first two axes in the PCA for each upper-lower petal surface pair for each species, which shows how different the chroma of upper and lower surfaces are for each species. We then did a Wilcoxon rank sum test to assess whether the chroma differences between upper and lower petal surfaces are more pronounced for closing than non-closing daisies.

Do less apparent lower petal surfaces result in lower herbivory rates?

To determine whether herbivores are less likely to eat closed flowers with cryptic lower petal surfaces than open flowers or closed flowers with bright lower surfaces, two separate experiments were conducted with angulate tortoises (*Chersina angulata*), a native herbivore. Experiments were conducted between 9 am and 3 pm on warm days (>20°C).

For the first experiment, ten tortoises were presented with six arrays consisting of ten flowers placed on edible Asteraceae leaves. Arrays were presented to each tortoise across a five hour period to prevent tortoises from becoming satiated, and arrays were placed 30-50 cm from each tortoise. Tortoises were left alone for at least 15 minutes after each trial. The arrays consisted of

(1) open *Dimorphotheca pluvialis* flowers, (2) closed *D. pluvialis* flowers (cryptic lower surface), (3) open *Arctotheca calendula* flowers, (4) closed *A. calendula* flowers (cryptic lower surface), (5) open *Ursinia calenduliflora* flowers, and (6) closed *U. calenduliflora* flowers (bright lower surface) (see Fig. 4.1). "Closed" flowers were kept in a cold, dark cooler until used to prevent them from opening. The sequence of the presentation of the arrays was randomized for each tortoise, and only four tortoises were exposed to arrays 5 & 6 due to limited availability of *Ursinia* flowers. Each trial ended when the tortoise walked away, or when approximately 75% of the leaves were eaten. A flower or leaf was recorded as "eaten" when any part of it was consumed. If a tortoise was not interested in a particular array, the array was retried later in the day or the next day. To assess whether the state of flowers (i.e. open or closed) influenced herbivore (i.e. tortoises) choices to eat flowers or leaves, we used the data from the first experiment. We conducted mixed effect models (LMM) with a binomial distribution and logit link function. We used a tortoise's sequential choice between flowers and leaves as the response variable, and the flower state (open/closed) as a fixed predictor variable. We specified tortoise individual as random intercept to control for the non-independent correlation structure of the data. To control for the effect that the difference in open flower size, closed flower size, and leaf area might have on encounter rates, we added weights to the analysis. The leaf area in trials with open flowers was twice as much as the flower area, and the leaf area in trials with closed flowers was four times as much as the flower area. We ran a separate LMM for each plant species. Analyses were conducted using the 'lme4' package in R.

The second experiment controlled for the difference in visual signal size of open and closed flowers, and thus explicitly testing the effect of colour. Two arrays were presented sequentially to fifteen tortoises. Each array consisted of ten open *A. calendula* flowers on Asteraceae leaves,

where flowers were placed upright in the first array (i.e. upper petal surface exposed) and upside down in the second (i.e .lower petal surface exposed). Trials were conducted as above. We conducted a binomial LMM to test whether colour (as presented by upper and lower petal surfaces of open flowers) influenced tortoises' preferences to eat flowers or leaves. We again used tortoise choice between flowers and leaves as response variable, and we used orientation (i.e. upright versus upside down; a proxy for colour) as predictor variable, whilst specifying tortoise identity to control for data non-independence and controlling for leaf and flower size.

Results

Are lower petal surfaces of closing daisies less apparent to herbivores than upper petal surfaces?

The difference in visibility between upper and lower petals surfaces within species (ΔJND) was higher in closing daisies than non-closing daisies for herbivore vision (Fig. 4.3), for chromatic ΔJND (horse: $W = 445$, $p = 0.02$; goat: $W = 416$, $p = 0.008$; chameleon: $W = 488$, $p = 0.06$), and achromatic ΔJND (horse: $W = 435$, $p = 0.01$; goat: $W = 383$, $p = 0.003$; chameleon: $W = 312$, $p < 0.001$). This shows that non-closing daisies do not have markedly different upper and lower petal surfaces, whereas closing daisies showed strong differences between these surfaces.

Once we controlled for phylogeny, all effects in the above analyses became non-significant.

Closing daisies had less visible lower surfaces than non-closing daisies for ungulate vision, but not for chameleon vision (Table 4.1, Fig. 4.3).

Do closing daisies produce different colours on their upper and lower petal surfaces?

Closing daisies had larger differences in colour between upper and lower petal surfaces than non-closing daisies ($W = 987$, $p = 0.002$, Fig. 4.2). The mean difference in colour between upper and lower petal surfaces for closing daisies was 0.07 (mean = 0.08) and for non-closing daisies was 0.03 (median = 0), measured as Euclidean distance on the first two axes of a PCA of reflectance spectra inflection points.

Do less apparent lower petal surfaces result in lower herbivory rates?

LMMs showed that tortoises foraged selectively on flowers rather than leaves when flowers were open, but not when flowers were closed, for *A. calendula* ($z = 4.080$, $p < 0.001$)and *D. pluvialis* ($z = 4.337$, $p < 0.001$), but not for *U. calenduliflora* ($z = 0.935$, $p = 0.35$, Fig. 4.4).

When open flowers with cryptic lower surfaces were placed upright (upper surface visible) and upside down (lower surface visible) to control for the size of the signal when testing herbivory rates, tortoises foraged selectively on flowers rather than leaves when flowers were upright, but not when flowers were upside down ($z = 7.969$, $p < 0.001$, Fig. 4.4).

Discussion

Our study shows that Namaqualand Asteraceae species that close their capitula during parts of the day have cryptic lower petal surface colouration that acts as an anti-herbivory mechanism. Lower petal surfaces of closing daisies are both less apparent than upper petal surfaces and produce different colours than upper surfaces, and this is not the case in non-closing daisies.

Additionally, lower petal surfaces of closing daisies are less apparent to herbivores than those of non-closing daisies. Together, this suggests that the differences between upper and lower petal surfaces in closing daisies did not arise solely due to selection by pollinators to increase the visibility of upper petal surfaces, but rather that the lower surface colouration of closing daisies is under selection to reduce herbivory. Our experiments with herbivores confirm this, clearly showing that open daisies are eaten more than closed daisies, and that this results from a difference in colouration of the visible petal surface.

Studies on floral trait evolution have mainly focused on the role of pollinators as agents of selection, with the selective effects of florivory receiving less consideration, despite clear fitness costs associated with loss of floral tissue (Strauss *et al.* 1996; Gómez 2003; Strauss and Whittall 2006; Kessler *et al.* 2010; Sõber *et al.* 2010). Thus, while cryptic flower colouration, as we have demonstrated here, may seem counterintuitive given that pollinators should select for apparency in flowers, the benefits of reduced florivory may outweigh the costs of reduced apparency to pollinators in some situations. Evolution of floral crypsis in response to selection by florivores is perhaps most likely when crypsis does not reduce apparency to pollinators. This is clearly the case in the nocturnally closing flowers that we have studied which effectively hide from herbivores, through a combination of flower closure and cryptic lower petal surface colouration, only during periods of the day when they are not signalling to pollinators by exposing their apparent upper petal surfaces. Similarly, plants that rely primarily on scent for pollinator attraction may not suffer any pollination costs of reducing visual apparency of their flowers. Several examples of putatively cryptic green or brown flowers with strong olfactory pollinator attraction are known, such as asclepiads (Shuttleworth and Johnson 2009) , *Satyrium microrrhynchum* (Johnson *et al.* 2007), and *Eucomis* (Shuttleworth and Johnson 2010).

However, to our knowledge, neither cryptic flower colouration nor its potential role in reducing florivory have been investigated in these systems. Another possibility is that flowers could be cryptic to florivores, but still highly apparent to pollinators if the wavelength sensitivities of their visual systems are different. While we are not aware of studies that have explored this possibility in the context of floral herbivory, the extensive research on the evolution of red flower colour in hummingbird pollinated plants provides an analogous example, where red flowers are detectable by effective hummingbird pollinators whilst being less detectable to more costly flower visitors, such as bees (Rodríguez-Gironés and Santamaría 2004; Shrestha *et al.* 2013; Bergamo *et al.* 2016; Rivest *et al.* 2017). Cryptic colouration as anti-herbivore defence in plant vegetative tissue is increasingly being recognized (Niu *et al.,* in press). Various studies have shown local adaptation of leaf colour to match the background colour, particularly in sparse vegetation or in rocky areas, leading to reduced herbivory (Strauss and Cacho 2013; Niu *et al.* 2014; Strauss *et al.* 2015). For instance, some palatable plant species on scree slopes match the colour of the scree, and the lower apparency reduces herbivory rates (Strauss and Cacho 2013; Niu *et al.* 2014). Our results suggest that cryptic colouration might also be present in floral tissue, and not only vegetative tissue.

Chemical defence of flowers represents an alternative mechanism by which plants can potentially overcome the trade-off they experience between floral apparency to pollinators and florivores. Like visual crypsis, floral chemical defence is likely to evolve in response to florivore selection only when it does not carry a strong pollination cost, i.e. when it does not deter pollinators. In some cases, chemical cues can act as both deterrent to herbivores and attractant of pollinators, as seen in carrion-mimicking flowers (Lev-Yadun 2014), much in the same way as red coloured flowers that attract bird pollinators and avoid pollen-feeding insects (Rodríguez-

Gironés and Santamaría 2004; Shrestha *et al.* 2013; Bergamo *et al.* 2016; Rivest *et al.* 2017). Alternately, in the same way that decreased floral visual apparency decreases herbivory and potentially alters pollination rates or pollinator identity, chemical defences can alter floral attractiveness to pollinators (Zangerl and Berenbaum 2009). For instance, in *Nicotiana* species, defensive compounds are correlated across vegetative tissues and nectar, and selfing species have much higher defensive compound levels than outcrossing species, suggesting that the optimal level of chemical defence is not expressed when pollination interactions are important (Adler *et al.* 2012). In our study, herbivory rates of closing flowers with bright lower petal surfaces (i.e. *Ursinia calenduliflora*) were low under all treatments in our experiments, and we also observed this during preliminary trials for *Felicia* species (recurving petals). One possible explanation is that some daisy species may have chemical defences against herbivory, and that some pollinators are not deterred by these compounds. The Asteraceae are well-known for containing many species that are toxic to both invertebrates and vertebrates (Milton 1992; Pelser *et al.* 2005). This supports Strauss et al. (2015) that showed cryptic species might be more palatable than apparent species, and that apparent species have higher chemical defences. The probability that either chemical defence or visual crypsis evolves as anti-herbivore defence thus likely depends on the chemicals which a plant can produce and whether these chemical negatively influence pollinator interactions.

Nocturnal closing of flowers in Asteraceae is conserved at the genus-level (Stirton 1983), and once we controlled for phylogeny in our surface visibility analyses, all effects became non-significant. Because closing is conserved phylogenetically and selection strongly favours the association of closing and cryptic lower petals only, selection for cryptic colouration on lower surfaces would result in a signal of conservatism of colour, even if the trait is genetically labile.

Unfortunately, our data have few phylogenetically independent contrasts, and we ultimately sampled four clades in which all species close, and three clades in which all remain open. Our experimental trials, involving species from three unrelated genera suggest an adaptive function for cryptic colouration, which likely shows that cryptic colouration is strongly selected for rather than genetically linked to petal movement. The solution to the low phylogenetic power would be to sample lineages outside the Asteraceae, and to verify that our trends are consistent in other lineages. Particularly, we have observed that similar cryptic abaxial surface colouration is present in Oxalidaceae, Iridaceae and Scrophulariaceae, families with closing flowers.

The JND values in our results are often well above 1 (the standard cut-off for distinguishability), and this probably results from the lack of accurate information on the visual systems of the appropriate herbivores. Garcia *et al*. (2017) recently investigated differences in discrimination abilities between closely related bee species, and showed high levels of variation. They advise that unless behavioural data for a particular species is available, all results from the RNL model should be interpreted as relative differences within a focal species. That is, due to the model's sensitivity to receptor noise levels and the high variance of this between species, we cannot know whether JND values above 1 are visible to a species if we do not have the correct receptor-noise information, but values that are relatively higher or lower than one another can be interpreted as relatively more or less visible to that observer species. Thus, we can compare the relative surface differences between spectra within an observer species' model, but not across the different herbivore species.

Conclusion

By combining herbivore visual modelling and herbivory experiments, we show that abaxial flower surfaces are less visible to ungulate and reptilian herbivores than adaxial surfaces in closing daisies, and that this translates into lower herbivory rates. Reducing the loss of flowers may be particularly important in annual species with long-lived flowers, and decreasing visibility when flowers are closed might have fewer trade-offs than chemical defences. Camouflage in plants remains an understudied phenomenon, and here we show that crypsis may be an effective defence strategy for floral tissue during times when pollinators are inactive.

Tables

Table 4.1. One-tailed Wilcoxon rank sum tests showed that lower petal surfaces of closing daisies are less visible than lower surfaces of non-closing daisies in ungulate, but not chameleon, vision.

Visual system	Chromatic		Achromatic	
	W	p	W	p
Horse	902	0.005	870	0.01
Goat	918	0.003	852	0.02
Chameleon	673	0.46	797	0.07

Figures

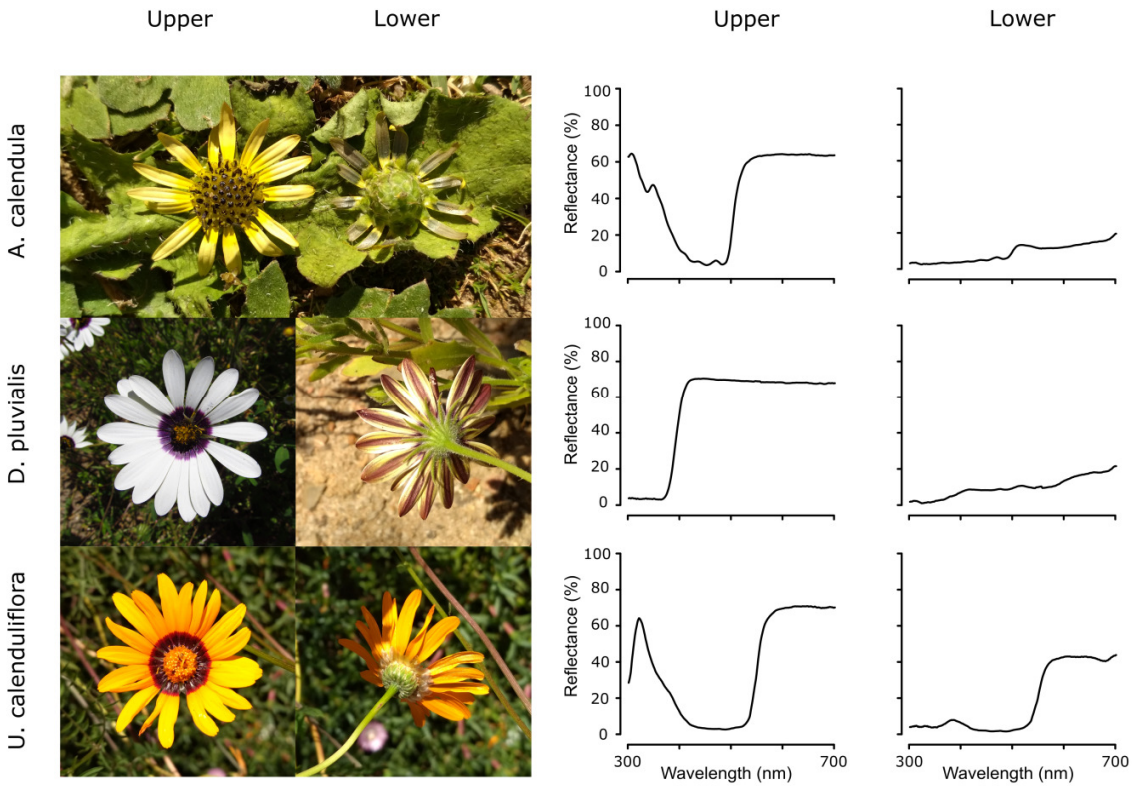

Figure 4.1. The three species, and their associated reflectance spectra, used in the herbivory experiments are shown. The leaves shown with *Arctotheca calendula* are the ones used in the experiments. *Arctotheca calendula* and *Dimorphotheca pluvialis* have cryptic lower petal surfaces, whereas *Ursinia calenduliflora* has a bright lower petal surface. All three species close at night.

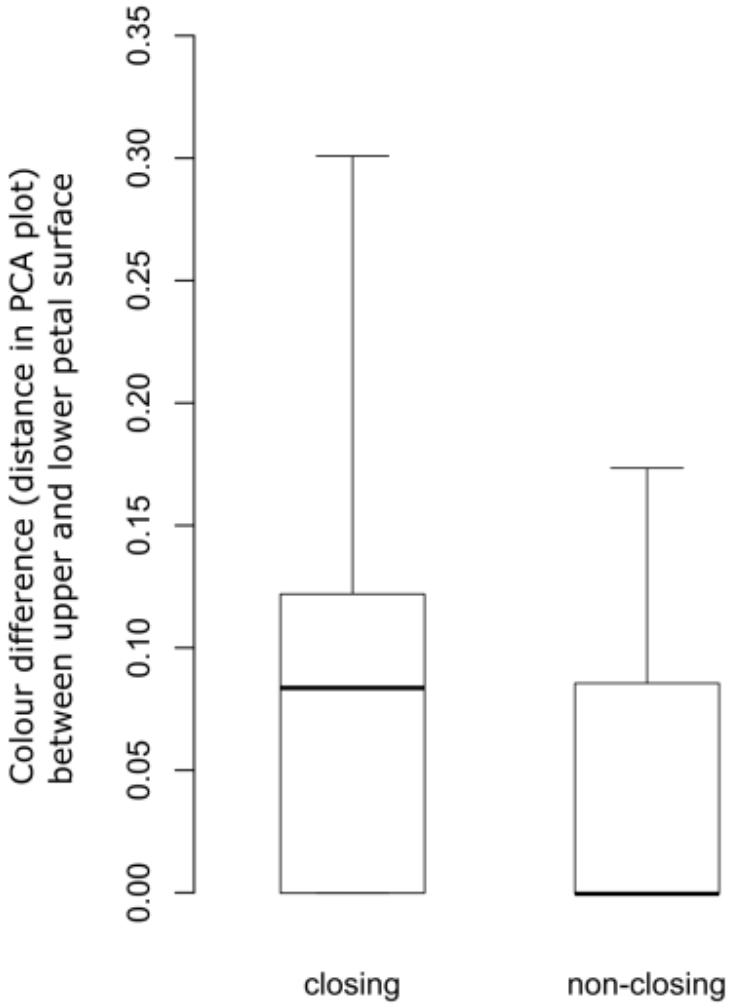

Figure 4.2. The difference in colour between upper and lower petal surfaces was determined for each species by calculating inflection points on reflectance spectra. Inflection points were used in a PCA, and Euclidean distances between upper-lower surface pairs were calculated for each species. The further away points in the PCA were from one another, the more different the colours were. We then tested whether closing daisies showed a larger difference in colour

between upper and lower petal surfaces than non-closing daisies, as expected when different selection pressures are operating on upper and lower petal surfaces in closing daisies.

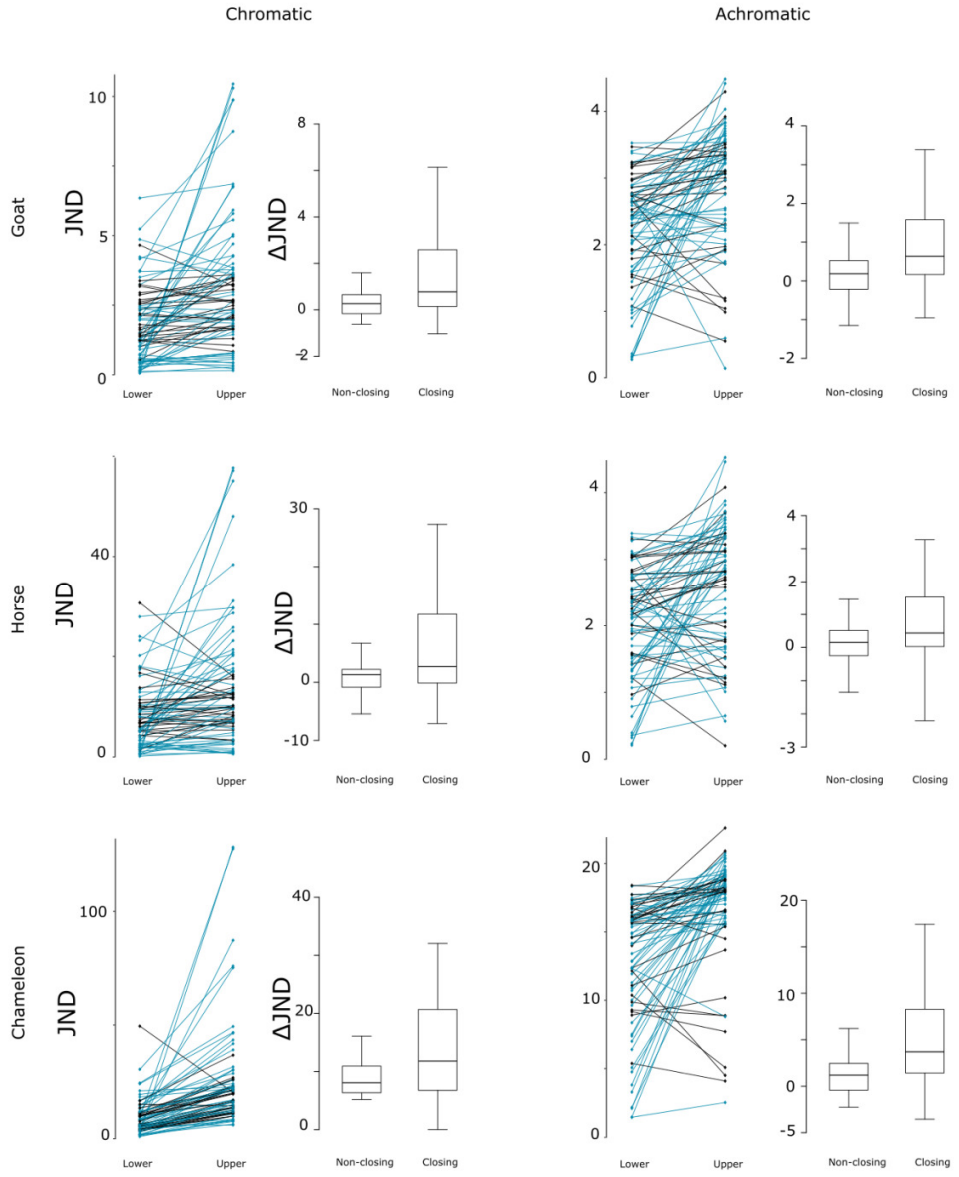

Figure 4.3. Just noticeable differences (JNDs) calculated from the receptor noise limited model are shown for ungulate (goat and horse) and chameleon (as proxy for tortoise) chromatic and

achromatic vision. For each set of two graphs, the first depicts the JND for lower and upper petal surfaces for each species (linked by lines) quantified against a standard green background. Black lines represent non-closing species and blue lines show closing species. The second graph in each set of graphs shows the median difference (and quartiles as whiskers) in JND (i.e. ΔJND) between upper and lower surfaces for closing and non-closing species. The closer the ΔJND is to zero, the smaller the difference in apparency between the upper and lower petal surfaces.

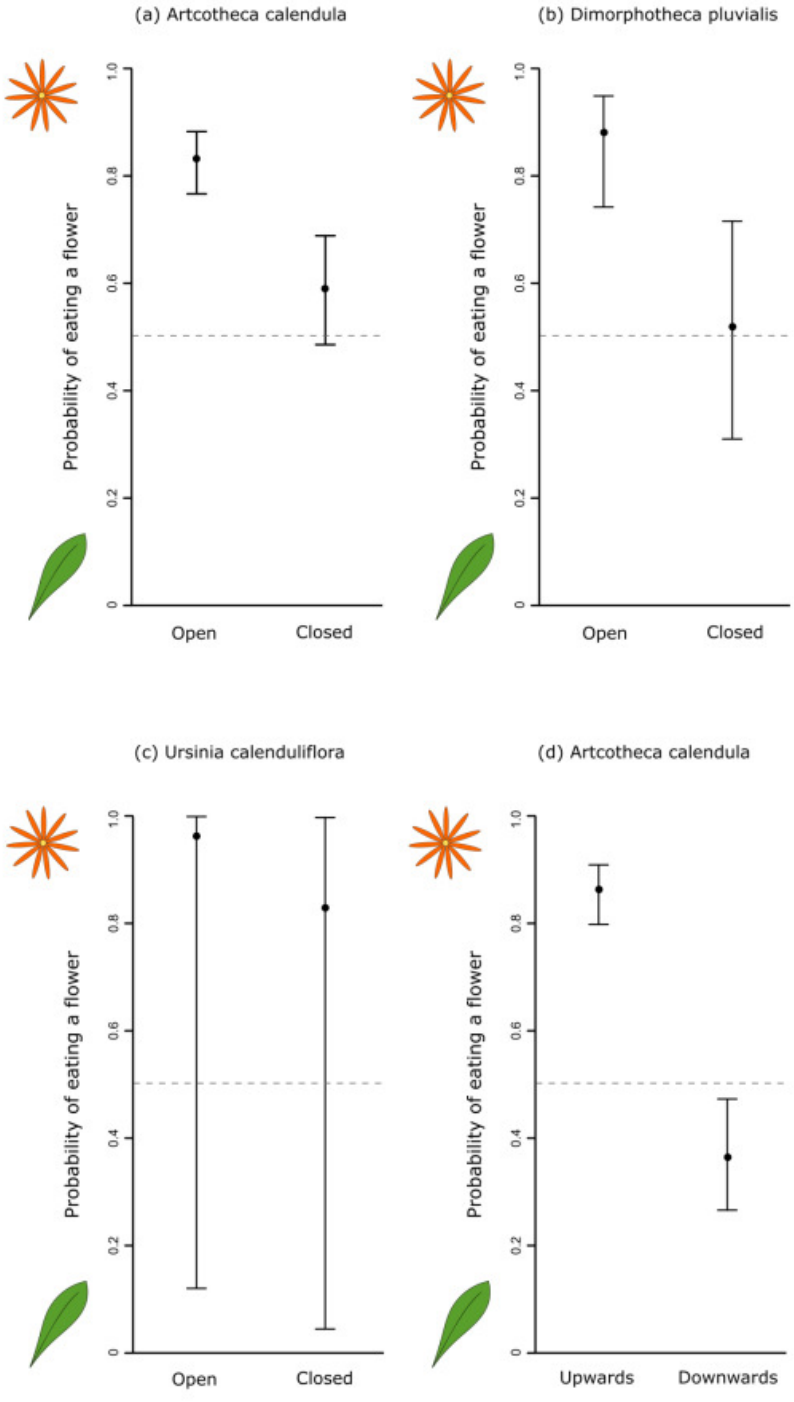

Figure 4.4. Tortoises were presented with sequential arrays containing 10 flowers placed on edible leaves. Tortoises ate more flowers than leaves when flowers were open for (a) *Arctotheca*

calendula and (b) *Dimorphotheca pluvialis*, which had cryptic lower petal surfaces, but when flowers were closed, tortoises foraged randomly. Tortoises showed no preference for open or closed flowers over leaves for (c) *Ursinia calenduliflora*, which had non-cryptic lower petal surfaces. (d) Further, we controlled for visual signal size by conducting the same trials using open *A. calendula* flowers that were placed upright or upside down on leaves. Tortoises selectively ate flowers rather than leaves when flowers were upright, and selectively ate leaves when the cryptic lower surface was exposed (i.e. upside down). Graphs show least square means from LMMs. When error bars overlap the 0.5-line, the tortoises showed no selectivity for either flowers or leaves (i.e. they foraged randomly). High values indicate a preference for flowers, whereas low values indicate a preference for leaves.

Chapter 5

Fundamental and realized pollination niche breadths in Namaqualand daisies across a community diversity gradient

Jurene E. Kemp & Allan G. Ellis

Abstract

Plants are often only visited by a subset of the available pollinator species in a community. This can either result from evolved phenotypic plant traits that influence which animals act as pollinators (i.e. the fundamental pollination niche), or from contemporary ecological interactions (i.e. the realized pollination niche). Particularly, floral symmetry is often invoked as a good indicator of pollination niche breadths, with actinomorphic species viewed as generalists. In Namaqualand, South Africa, diverse communities of functionally actinomorphic annual daisy species flower en-masse at high densities. In this competitive context, we might expect ecological specialization of daisies despite floral symmetry remaining conserved. Here, we quantify ecological specialization and test the extent to which it reflects fundamental or realized pollination niches.

We first quantified ecological specialization across daisy communities by constructing pollinator visitation networks and tested whether Namaqualand daisies are more specialized than actinomorphic species from other regions. We used two approaches to determine whether ecological specialization represents narrow fundamental niches or whether it results from contemporary ecological interactions. First, we tested whether pollination niche breadths were associated with floral signalling and reward-related traits, which is expected if ecological specialization represents fundamental niches. Next, we used two *Gorteria diffusa* morphs in a transplant experiment where we manipulated plant competitor densities across a pollinator diversity gradient to test whether niche breadths are influenced by community context, as expected if ecological specialization reflects realized niches.

We found that most daisy species interact with fewer pollinator species than expected from random interaction, but that Namaqualand daisies did not interact with fewer pollinators than actinomorphic species from other parts of the world. We showed that various floral traits, such as longer nectar tubes and complex flower colour patterns, were associated with narrow pollination niches. Moreover, one *Gorteria diffusa* morphotype exhibited a narrow fundamental pollination niche that persisted regardless of changes in the surrounding pollinator community or changes in competitor density.

Our results challenge the traditional view that functionally actinomorphic species have broad pollination niches. Particularly, our results suggest that many Namaqualand daisies exhibit ecological specialization, and that in some species, this represents narrow fundamental pollination niches. Importantly, our results suggest that quantitative, rather than qualitative differences in pollinator attraction are prevalent, and that niche breadths can potentially be overestimated if relative interaction frequencies are ignored. We suggest that, despite the conserved actinomorphic floral symmetry of daisies, under certain conditions adaptations to visual signal and reward-related traits might arise that result in ecological specialization.

Introduction

The extent to which plants are specialized in their pollination interactions is a hotly debated question in pollination biology (Waser *et al.* 1996; Johnson 2010), and plant species within communities typically exhibit a range of pollination niche breadths (Bascompte *et al.* 2003; Armbruster 2017). The fundamental pollination niche that a species occupies is determined through both evolutionary adaptations and genetic constraints (Poisot *et al.* 2011). However, the pollination niche actually exploited by plant populations (i.e. the realized niche) likely departs from this fundamental niche in two main ways. First, a plant species might use all of the pollinators comprising its fundamental niche that are available, but turnover in available pollinators can result in variation in pollinator usage across communities (i.e. each population exploits a different part of the fundamental niche). Here the restriction of the pollinator niche results from spatial variation in pollinator occurrences, and not from ecological interactions between plants. Alternatively, a plant species might only use a subset of its available potential pollinators (i.e. a subset of its fundamental pollination niche) in a community because of the presence of co-occuring plant species that are superior competitors. Here, ecological interactions, rather than pollinator availability, constrain the realized niche.

If ecological specialization (i.e. when plant species use a subset of the locally available pollination resource – Armbruster 2017) is dependent on the community context, interaction partners may vary geographically and plants may use a larger subset of their fundamental pollination niche in low competition environments (Pauw 2013). Consequently, plants can potentially become locally adapted to the pollinators they use at a site, and this is then often reflected in adaptive phenotypic traits associated with narrower fundamental pollination niches. Specialization of plants to pollinators can result from selection for increased conspecific pollen

transfer efficiency (through better morphological fit between participants, or through increased attractiveness to pollinators and thus higher visitation rates) (Grant and Grant 1965; Stebbins 1970), or from selection to reduce reward exploitation and floral damage (Lunau *et al.* 2011; Santamaría & Rodríguez-Gironés 2015; Bergamo *et al.* 2016). Specialization can also result from selection to reduce heterospecific pollen transfer and pollen discounting (Sargent and Otto 2006). Alternately, if pollinator visitation rates are low, phenotypic traits that allow pollination by multiple species might develop (see Aigner 2001) and plant species can be adapted to be generalist.

Plants can thus become phenotypically specialized when evolving floral traits, such as particular scents (Theis *et al.* 2007; Raguso and Weiss 2015), shapes (Johnson *et al.* 2001; Alexandersson and Johnson 2002; Pauw *et al.* 2009), or rewards (Johnson *et al.* 2006; Pauw 2006), that limit the number of pollinator species that visit a plant species (Ollerton *et al.* 2007; Armbruster 2017). For instance, olfactory cues exuded by flowers can both attract mutualists and deter antagonists, resulting in specialized interactions (Kessler *et al.* 2013). Similarly, flower colour can attract some pollinator groups and exclude those with different visual systems (Lunau *et al.* 2011; Santamaría & Rodríguez-Gironés 2015; Bergamo *et al.* 2016). Morphological traits, such as nectar tube length, can limit the number of species that are able to access rewards or that make contact with reproductive parts to pollinate a flower (Newman *et al.* 2014; Anderson *et al.* 2014). Particularly, floral symmetry is often invoked as a trait associated with ecological specialization (Neal *et al.* 1998; Fenster *et al.* 2004; Gómez *et al.* 2006), as bilaterally symmetric flowers are more likely to exclude certain visitors from accessing rewards (Ostler and Harper 1978; Neal *et al.* 1998; Sargent 2004) and are more likely to have precise pollen placement by dictating pollinator movement within the flower (Sprengel 1793, Neal *et al.* 1998, Sargent 2004).

However, floral symmetry is not always a good predictor of ecological specialization.. Triggerplants, for example, are strongly zygomorphic but are visited by many pollinator species (Armbruster *et al.* 2009; Armbruster 2017). Species with conserved actinomorphic floral symmetry might not be able to develop traits that improve the placement of pollen on pollinator bodies, and selection should thus rather act on reward or visual signal traits that influence the visitation rates of various pollinator species. Selection on traits to increase visitation rates of efficient pollinators or decrease visitation rates of costly pollinators should result in narrow fundamental pollination niches (but see Aigner 2001), even if flower symmetry remains unchanged. However, in actinomorphic plant species, narrow observed pollination niches are often assumed to be realized niche breadths that result from plant competition or pollinator availability, rather than reflecting a narrow fundamental niche.

The extent to which ecological specialization reflects the fundamental, rather than realized, pollination niche is often difficult to assess. The recent surge in sampling bipartite interaction networks, for example, has allowed characterization of the range of pollination niche breadths occurring within communities, but these studies generally do not consider whether the observed niche represents the fundamental niche, and do not verify niche breadths with experimental tests (but see Spiesman and Gratton (2016) , Junker *et al.* (2010), and Augustyn *et al.* (2016) for herbivorous insects). An effective way to test the breadth of the fundamental pollination niche is to conduct transplant experiments and expose plant species to different pollinator communities. This has been done for some phenotypically specialized species (see work on South African flora with long nectar tubes - e.g. Alexandersson and Johnson 2002; Waterman *et al.* 2011; Anderson *et al.* 2014; Newman *et al.* 2015), but is rarely done for actinomorphic species which are assumed to be generalist in their interactions. Transplant experiments are, however, usually done

with few plant individuals, and fundamental niches might be underestimated if potential pollinators are not provided with enough time or reward incentive to learn to interact with the novel plant species.

Here we determine whether actinomorphic species that flower at high densities among many heterospecifics exhibit narrow pollination niches, and we assess whether these represent fundamental or realized niches. If observed pollination niche breadths are evolved (i.e. fundamental), we expect visual signal or reward-related traits to be associated with niche breadth, and we expect niche breadths and pollinator identity to be consistent across community contexts. Particularly, we investigate pollination niche breadths in daisies during a mass flowering display in Namaqualand, South Africa, where hundreds of species flower simultaneously at high densities. Although daisy flowerheads consist of actinomorphic disk florets and zygomorphic ray florets, the flowerhead is functionally actinomorphic, and we consider daisies to be actinomorphic throughout. The high heterospecific densities could result in narrow realized pollination niches through plant competition, and previous work has shown that heterospecific interference has a negative effect on the fecundity of these daisies (De Waal *et al.* 2015). To test whether ecological specialization in Namaqualand daisies is evolved (i.e. fundamental niches) or the result of ecological context (i.e. realized niches), we focus on two geographically closely situated areas with different plant and pollinator community compositions. Particularly, each of these two areas contains a different floral morph of *Gorteria diffusa*, and we use this polymorphism in transplant experiments to assess whether plant specificity remains constant when plants are exposed to a different pollinator community whilst varying interspecific plant competition.

We thus address four main questions:

1) Do actinomorphic daisy species exhibit ecological specialization (i.e. exploit a subset of the available daisy visiting pollinator fauna)?

2) Are Namaqualand daisy pollination niche breadths comparable to actinomorphic species elsewhere in the world?

3) Are narrow pollination niche breadths associated with floral traits, as expected from functional specialization?

4) Are pollination niche breadths influenced by commuity context, as expected when ecological specialization represents realized niches?

Methods

All data were analysed in R (R core team, 2016).

Study system

The Succulent Karoo region of South Africa hosts more than 400 Asteraceae species (Snijman 2013). The short flowering period (largely confined to the austral spring), low incidence of selfing (De Waal *et al.* 2014), and high number of annual species suggest that pollinator interactions may be critical for plant persistence. However, limited work on the pollination systems of these daisies has suggested that pollinator abundances are low (Struck 1994), which

leads to the expectation that selection to increase the effectiveness of pollinator interactions may be prevalent. Previous work on Namaqualand daisies has shown that selection by both pollinators (Ellis and Johnson 2009; De Jager and Ellis 2012, 2013) and florivores (De Jager and Ellis 2014) influence floral phenotype. If some floral phenotypes are preferred or avoided by certain insect species, we might observe narrow pollination niches.

Do actinomorphic plant species exhibit ecological specialization?

Flower visitation networks were constructed in August and September 2015 for six sites which represent two broad communities. The two community types vary in composition (Chapter 2, Kemp *et al*. in press). The first community (referred to as 'Kamies') has high flower densities, with high plant and insect species richness. The second community (referred to as 'Soebats') has lower flower densities, and fewer plant and insect species. Flower visitor observations were conducted for a hundred $1m^2$ plots (located within a 1 ha site) at each of the six sites. Each plot was observed for 15 minutes between 10 am and 4 pm when flowers were open and pollinators were active (i.e. 25 observation hours per site, and 150 observation hours in total). The number of open flowers for each plant species and insect visitation frequencies to the various plant species were recorded during each observation period. Insect visitors were caught, and identified to species where possible (otherwise sorted to morphospecies). For each site (n = 6), we calculated the total number of insect visitors to each plant species and we calculated the number of flowers observed for 15 minutes for each plant species. We used this to determine the number of visits per flower per 15 minute interval for each insect species to each plant species, and we multiplied these visitation rate values by 1000 to create integers. Plant species that occurred in fewer than 20 observation periods at a site were excluded from all analyses.

For each of the six networks, we calculated ecological specialization of plant species as interaction partner richness, which is the number of interaction partners a species has. To account for the highly uneven distribution of visits across pollinator species, we used Hill numbers of the Shannon diversity index (i.e. "d" in the 'vegetarian' package in R) as an additional specificity metric that we refer to as interaction partner diversity. This measure calculates the number of interaction partners each plant species has, and weights it with the visitation rates from the respective insect partner species. Thus, if a plant species is visited by many insect species, but is visited disproportionately more by one species, this metric will show that the plant species is effectively specialized. For both indices, high values indicate generalization. To assess whether observed niche breadths resulted from random visitation by pollinators, we compared our observed specialization values to expected values from randomized matrices. We randomized each of the six networks 999 times using the 'permatfull' function in the 'vegan' package (Oksanen *et al.* 2016), and then calculated the distribution of both interaction partner diversity and richness from them for each plant species/network combination. We used z-scores to determine whether observed niche breadths deviated from random. The number of visits each insect species made at a site was kept constant, but individual insects were allowed to randomly interact with any plant species at a site. We used the number of visits scaled by flower abundances (i.e. visits per flower; calculated above) to control for plant density effects. We thus assumed equal abundances of plant species, and in our null matrices, insect visits were randomly assigned to any plant species, which is what we expect if insects are randomly visiting plant species.

Are Namaqualand daisy pollination niche breadths comparable to actinomorphic species elsewhere in the world?

We assessed whether pollination niche breadths of Namaqualand daisies are similar to those of other actinomorphic species and lower than those of zygomorphic species, as expected when floral symmetry is associated with pollination specialization.

To do this, we calculated interaction partner richness and diversity for plant species in previously sampled networks available on the Interaction Web Database. We excluded networks that only considered one pollinator group (such as hummingbirds), or that looked at invasive or restoration systems. We then classified floral symmetry (actinomorphic or zygomorphic) of each plant species in these networks and separated daisies from other actinomorphic species [Appendix 2 Table S5.1]. We used a linear mixed effect model (LMM) in the "lme4" package, with "network" as random factor (intercept), to assess whether interaction richness and diversity vary between zygomorphic species (global data set), actinomorphic species (excluding daisies; all global data set), non-Namaqualand daisies, and Namaqualand daisies.

Are floral traits (i.e. visual signals or reward availability) of actinomorphic plants associated with pollination niche breadths, as expected from functional specialization?

To assess whether particular floral traits are associated with ecological specialization, we quantified various traits associated with visual signalling and rewards that could potentially influence pollinator preferences and foraging behaviour.

Visual signals included dominant flower colour (explained below), flower colour signal complexity (explained below), and total signal size (i.e. the average number of inflorescences per

plant multiplied by the average inflorescence size). For reward-related traits, we measured nectar tube length, which affects the accessibility of nectar rewards, and we measured the number of florets presenting pollen per day, which we use as proxy for the amount of pollen reward available.

To quantify colour signal complexity and dominant flower colour, flower reflectance spectra between 300 and 700 nm were collected for all annual and perennial daisy species occurring at the six sites. For polymorphic species, the different phenotypes were sampled separately. Spectra were recorded indoors at a 45° angle using an OceanOptics USB4000 Spectrometer calibrated with a diffuse reflectance WS-2 white standard.

To quantify dominant flower colour, plant species were grouped together based on inflection points of the spectral curves of the outer ray florets (see Kemp *et al*., in press). We used the groupings identified in Chapter 2, and species without ray florets were placed in a separate group. The outer ray is the widest colour band in the daisy inflorescence (see Chapter 2) and thus represents the dominant flower colour. Plant species belonged to eight colour groupings.

Many of these Asteraceae species exhibit concentric colour rings, often referred to as a bulls-eye pattern, which create multiple contrasts within each daisy flowerhead. To account for this, we measured reflectance spectra for three sections of the daisy inflorescence; namely, the disk florets, inner ray florets and outer ray florets (see Chapter 2 for details). We use the number of colour contrasts between these three sections as a measure of flower colour signal complexity. In order to assess whether the colours between the adjacent sections were the same or different, we used the colour categories identified in Chapter 2 that were based on inflection points. For instance, if an inflorescence consisted of yellow disk florets, black inner ray florets and orange

outer rays, two levels of contrast would be present in the inflorescence, whereas inflorescences that are entirely yellow would have zero levels of contrast. In species where flowerheads only consisted of disk florets, zero contrast levels were present.

To determine whether floral traits are associated with ecological specialization, we used four linear models to assess the effects of dominant flower colour (8 categories), flower colour complexity (3 categories), total visual signal size, nectar tube length and the number of florets presenting pollen on (1) interaction diversity, (2) interaction richness, (3) interaction partner functional group richness, and (4) interaction partner functional group diversity respectively. Data were log-transformed to improve normality where necessary. Insect species were assigned to the following functional groups: florivorous beetles (Coleoptera, mainly Meloidae), embedding beetles (mainly Scarabidae), non-embedding monkeybeetles (Scarabidae), bees (Apoidae), small flies (<1 cm from head to abdomen) (Diptera, mainly Bombyliidae, Tabanidae), large flies (Diptera, mainly Bombyliidae), and butterflies (Lepidoptera) [Appendix 2 Fig. S5.1]. We chose these groupings based on foraging behaviour (e.g. feeding on petals, ovules, pollen or nectar; highly territorial or not) and visual system (e.g. bees have trichromatic vision while flies have tetravariant vision).

Does ecological specialization in actinomorphic species represent the fundamental pollination niche, or a subset thereof that is influenced by community context?

To assess whether ecological specialization represents the fundamental niche or whether it is influenced by the ecological community context (i.e. realized niche), we conducted transplant experiments between the Kamies and Soebats community types (i.e. to alter the insect community plants are exposed to) while varying competitor plant densities (i.e. to alter plant

competition). We focused on two separate morphs of *Gorteria diffusa*, one naturally occurring at Kamies (i.e. the 'cal' morph) and one naturally occurring at Soebats (i.e. the 'soeb' morph). *G. diffusa* is an annual, self-incompatible daisy which displays 14 geographically structured flower morphotypes (Ellis and Johnson 2009). The transplant experiment was conducted during August and September 2016, and we decided to use *G. diffusa* morphs for transplants based on the results from the networks sampled in 2015.

If a *G. diffusa* morph has a narrow fundamental pollination niche, then we expect it to use few pollinator species, as well as the same pollinator species, irrespective of community context. If ecological specialization is dependent on the local community context, then we expect visitation rates and the pollination niche breadth to vary with changes in interspecific plant competition or with variation in the pollinator community. We first assessed this using the interaction networks by calculating the turnover in interaction partners between sites within a broad community for the cal and soeb morphs respectively. For this, we used the Horn similarity index in the "vegetarian" package in R.

To experimentally test this, we constructed artificial two-species communities at both Kamies and Soebats. At both sites, twelve 1.5 x 1.5 m arrays were assembled, each containing nine *G. diffusa* individual plants (Fig. 5.1) and individuals of *Dimorphotheca sinuata* (a competitor with overlapping pollination niche). Three replicates of each of the following treatments were assembled: (1) low competition soeb, (2) high competition soeb, (3) low competition cal, and (4) high competition cal. Low competition treatments contained three *Dimorphotheca sinuata* individuals, and high competition treatments contained nine *D. sinuata* individuals. *Dimorphotheca sinuata* is one of few species that occurs in both communities, and it occurs in

particularly high abundances at Kamies. Low competition treatment species ratios were based on the relative densities of *D. sinuata* and Soeb at Soebats, whereas high competition treatment densities were chosen to give insects an equal choice between *D. sinuata* and *G. diffusa*. Each plant had multiple flowers (range: 2 – 10 flowers per individual). All daisy flowers were removed from the core transplant plots (1.5 x 1.5 m) and all *G. diffusa* flowers were removed from a 10 m buffer zone around the transplant arenas to prevent gene flow between transplanted and local plants.

Pollinator observations were conducted on a subset of plants in each array for 10 minute sessions. Each array was observed for at least 30 minutes in total, and each treatment was observed for at least 90 minutes at each site. In total, 1213 *G. diffusa* flowers were observed. To account for pollinators' inexperience with the introduced form, observations were not conducted on the first day. As 54 introduced *G. diffusa* plants (each with multiple flowers) were present at each site, we assume that pollinators encountered the introduced forms and became familiar with them.

Due to low visitation rates during the first experimental setup at Soebats, the experiments were repeated at Soebats to increase sample sizes and statistical power. We combine the data from the two Soebats experimental sessions for analyses. Experiments were not repeated spatially as spatial replication would require that additional plant communities would have been cleared to set up the experiments, and we wished to minimize our impact.

To determine whether ecological specialization represents realized or fundamental pollination niches, we used analysis of variance (ANOVA) to test the influence of morph (cal versus soeb), site and treatment (high versus low competition) in the transplant experiments on (1) interaction

partner richness, (2) interaction partner diversity and (3) pollinator visitation rate. If plant specificity is evolved, we expect no influence of site or competition treatment. We combined the data from the 10-minute observation periods for each array at each site, and used the separate arrays as replicates in the ANOVAs. For each array, we calculated the number of visits per flower (multiplied by 100 to create integers) for each insect species. We then calculated interaction partner richness and diversity, and the total number of visits each morph received in each array (i.e. visitation rate).

We also tested whether flower visitor identity varied for each floral morph between sites (Kamies versus Soebats). For this we calculated beta diversity (i.e. the turnover of species) in flower visitors to *G. diffusa* morphs using Horn similarity as advised by Jost (2007), where 1 indicates complete similarity (as expected under narrow fundamental niches) and 0 indicates complete change in visitor identity (as expected under broad fundamental niches). Beta diversity was calculated in the same way for functional groups.

Results

Do actinomorphic plant species exhibit ecological specialization?

For the interaction networks constructed in 2015, the Kamies community (i.e. consisting of three networks) consisted of 19 daisy species, 109 insect morphospecies, and 7811 interactions while the Soebats community consisted of 12 daisy species, 47 insect morphospecies and 3161 interactions (Fig. 5.2). Small flies were the most frequent interaction partner [Appendix 2 Fig.

S5.1]. The six networks sampled in 2015 showed that, on average 26.3% of potential interactions within a network were realized (s.d. = 0.06, Fig. 5.2). Fourteen plant species*site combinations were excluded from the data set due to low local densities.

The median number of interaction partners for plant species (i.e. richness) at Kamies was 7.5 (s.d. = 5.81) and 7.5 (s.d. = 4.41) at Soebats. Plant species' median weighted number of partner species (i.e. interaction diversity) at Kamies was 5.24 (s.d. = 3.10) and at Soebats was 2.64 (s.d. = 1.88). Median functional group interaction richness was 4 (s.d. = 1.70) at Kamies and 4 (s.d. = 1.41) at Soebats. Median functional group interaction diversity was 2.87 (s.d. = 1.26) at Kamies and 1.97 (s.d. = 0.96) at Soebats.

Observed specialization levels of most plant species were significantly higher than expected from random foraging null models for both interaction partner diversity (68 %) and interaction partner richness (55 %) (Table 5.1). Interestingly, some plant species showed variation between communities; such as *Dimorphotheca sinuata* (see Table 5.1). However, the majority of species showed consistent trends across communities.

Are Namaqualand daisy pollination niche breadths comparable to actinomorphic species elsewhere in the world?

Using global datasets, zygomorphic species had lower interaction partner richness than all actinomorphic groups (estimate = -1.80, t = -2.021, p = 0.04, Fig. 5.3). No differences in ecological specialization was found for interaction partner diversity (Fig. 5.3).

Are floral traits (i.e. visual signals or rewards) of actinomorphic plants associated with pollination niche breadths, as expected from functional specialization?

Interaction partner richness and diversity (based on insect species) was primarily influenced by flower colour traits; that is, dominant flower colour was associated with the number of interaction partners, plant species with one level of colour contrast were visited by fewer insect species than plant species with more or fewer contrasts, and plant species with larger visual signals (i.e. more and larger flowers) attracted more insect species and higher insect diversity (Table 5.2, Appendix 2 Fig. S5.2). Plant specificity was also related to floral rewards, and plant species with longer nectar tubes were visited by few insect species, and those with many florets presenting pollen were visited by many insect species (Table 5.2, Appendix 2 Fig. S5.2).

Does ecological specialization in actinomorphic species represent the fundamental pollination niche, or a subset thereof that is influenced by community context?

For the transplant experiments, cal plants at Kamies received 107 visits from 1 insect species, and soeb plants received 36 visits from 11 species. At Soebats, cal plants received 31 visits from 3 insect species and soeb plants received 35 visits from 14 insect species. Arrays with cal plants were observed for 480 minutes (8 hrs in total; 3 hrs at Kamies, 5 hrs at Soebats), and arrays with soeb plants were observed for 760 minutes (12 hrs 40 mins in total; 4 hrs 20 mins at Kamies, 8 hrs 20 mins at Soebats).

In the interaction networks, the cal morph was consistently visited by few insect species ($mean_{diversity}$ = 1.08, s.d. = 0.07) and the same insect species at each site ($Mean_{Horn\ similarity}$ = 0.99, s.d. = 0.005). In contrast, the soeb morph was visited by multiple insect species ($mean_{diversity}$ =

3.43, s.d. = 1.63), and these showed high turnover between sites ($Mean_{Horn\ similarity}$ = 0.59, s.d. = 0.28).

Interaction partner richness and diversity were always higher for soeb than cal morphs, but were not influenced by site or competitor density (Table 5.3). Visitation rates were lower at the Soebats site than at the Kamies site (Table 5.3).

Flower visitor identity in the transplant experiment remained consistent between sites for the cal morph for insect species (β = 0.95) and insect functional group (β = 0.97), whereas the soeb morph showed variation in visitor identity between sites for insect species (β = 0.23) but little variation in functional group (β = 0.78). At both Kamies and Soebats, the cal and soeb morphs shared few visitor species (β_{Kamies} = 0.16; $\beta_{Soebats}$ = 0.57) or functional groups (β_{Kamies} = 0.21; $\beta_{Soebats}$ = 0.63). However, because cal was always visited by the same insect species (i.e. *M. capensis*), the interaction beta diversity results indicate that cal is using a subset of the soeb morph's visitor assemblage.

Discussion

Namaqualand daisies have narrower pollination niche breadths than expected from random visitation, and many daisies effectively relied on less than two pollinator species (30% of sampled species). Interaction frequencies were highly uneven with many different insect species visiting a plant species, but few visitor species responsible for the majority of visits. We show that various floral traits are associated with pollination niche breadths, such as flower colour, flower pattern complexity, nectar tube length and the amount of pollen reward, which suggests a

functional association between plant traits and pollination niche breadths. Further, we show that one *Gorteria diffusa* morphotype exhibits a narrow fundamental pollination niche and consistently interacts with the same pollinator species across community contexts. Together, this suggests that many Namaqualand daisies exhibit ecological specialization, and that in some species, ecological specialization is evolved and not the result of community context.

Although our comparison to other global data sets supports the idea that actinomorphic flowers tend to be more generalist than zygomorphic species (Fenster *et al.* 2004; Gong and Huang 2009; Chamberlain *et al.* 2014), this did not hold true when the evenness of interactions was incorporated (i.e. using interaction partner diversity). This suggests that despite more insect species visiting actinomorphic plant species than zygomorphic species, actinomorphic species (including Namaqualand daisies) are effectively just as ecologically specialized as zygomorphic species. This potentially suggests that niche breadths of actinomorphic species might be overestimated when only assessing the number of interaction partners a plant species has without incorporating interaction evenness. Interestingly, Fenster *et al.* (2004) used the Robertson (1928) data set to show that more than half of the actinomorphic plant species assessed were pollinated by a single functional group, showing that many actinomorphic species are not generalist. Innate preferences for floral symmetry have been shown to vary between pollinator groups (Rodríguez *et al.* 2004; Wignall *et al.* 2006), and some pollinators favour actinomorphy over zygomorphy (Wignall *et al.* 2006).

We show that various plant visual signals (particularly flower colour and colour pattern complexity) and reward-related traits (i.e. nectar accessibility and pollen availability) are associated with ecological specialization. Because certain traits are consistently associated with

particular pollination niche breadths across community contexts, this suggests that ecological specialization might reflect fundamental niches, rather than realized niches. That is, visual signalling and reward-related traits may represent phenotypic specialization of flowers, where particular variants of these traits limit which pollinator species are attracted to flowers or which species can access rewards. Pollinator colour preferences has been shown to drive floral evolution (e.g. Newman *et al.* 2012; Shrestha *et al.* 2013, 2016; Russell *et al.* 2017, but see Cooley *et al.* 2008), but this is often coupled to morphological traits that improve pollen transfer (e.g. Junker *et al.* 2013). Here we show that although any insect can access the reproductive structures and act as pollinator for these daisies, plant species attract certain insects disproportionately more than others by potentially exploiting various foraging behaviours or visual preferences. Our results align with the findings of Mochizuki and Kawakita (2018) that showed dark red actinomorphic flowers of various plant families in Japan are primarily visited by fungus gnats, which also shows that narrow pollination niches in actinomorphic species can be associated with flower colour traits. Further, the association between floral traits and specificity also implies that certain traits are associated with broad fundamental niches, which suggests that some plant species might be adapted to be generalist (as discussed in Armbruster 2017, Aigner 2001).

Interestingly, plant species that interacted with few pollinator species had long nectar tubes. This phenomenon has repeatedly been shown for pollinators with long proboscides and is often the outcome of a coevolutionary arms race (e.g. Anderson and Johnson 2008; Pauw *et al.* 2009), but it has rarely been measured in shorter tubed plant species (but see Pyke 1982; Macior 1986; Pyke *et al.* 2012). It is, however, not unexpected that small insects can exert selection on nectar tube lengths. Although a limitation to the extent of nectar tube length evolution is imposed by the

body size of the pollinator, small insects can drive selection if the majority of visitors have short proboscides. For instance, the dominant pollinator at Kamies was *Megapalpus capensis*, a bee fly that has a body length of less than 10 mm and a proboscis of up to 12 mm (unpublished data). The *Gorteria diffusa* cal morph, that we show is visited almost exclusively by *M. capensis,* has a mean nectar tube length of 6.12 mm, which is one of the longest tube lengths we recorded (maximum: 7.17 mm for *Gazania leiopoides*). Other pollinators in Namaqualand, such as bee flies from the *Corsomyza* genus, have shorter proboscides and these species may be unable to feed on plant species which have adapted to *M. capensis*, resulting in plant specificity. The reduced pollination niche associated with longer nectar tubes can either result as a by-product of selection to increase the efficiency of the primary pollinator (Aigner 2001, 2004), or it can result from selection to exclude costly visitor species (Santamaría and Rodríguez-Gironés 2015). Our data are uninformative as to which of these processes are acting here. However, adaptations to improve the fit of pollinators and flowers are unlikely to develop in daisies due to the conserved inflorescence layout where anthers are evenly spread across the capitulum, and any insect that visits a daisy inflorescence will make contact with the reproductive structures. However, variation between the pollination efficiency of visitors can potentially select for excluding less effective pollinators from visitation. Although daisies cannot restrict access to pollen rewards, restricting access to nectar rewards might be sufficient to prevent certain insects from visiting.

Our transplant experiment shows that ecological specialization in the *Gorteria diffusa* cal morph reflects a narrow fundamental pollination niche that is not influenced by ecological context. Neither variance in competitor density nor differences in pollinator composition (represented by the two community types) influenced visitor identity, and the cal morph was consistently visited by *M. capensis.* The cal morph is thus entirely dependent on a single fly species for pollination

and potentially cannot exist outside the range of *M. capensis*. The high abundances of *M. capensis* during both years of our observations (and previous years - Chapter 3) and the high visitation rates at Kamies, suggest that it is a reliable pollination resource. The high occurrence predictability and high abundance of *M. capensis* may favour the exclusion of less efficient pollinators and antagonists; that is, high *M. capensis* visitation rates to the cal morph may increase pollen export efficiency (as argued by Ellis and Johnson 2010), and selection may favour the exclusion of some insects to reduce pollen loss and floral damage (Aigner 2001; Theis *et al.* 2007; Galen *et al.* 2011; Santamaría and MA Rodríguez-Gironés 2015).

In contrast, the *Gorteria diffusa* soeb morph showed variation in which pollinator species it used across communities, but not in richness or diversity of partners, suggesting that the soeb morph has a broad fundamental pollination niche and always attracts many pollinator species despite variance in community context. Annual variation in the pollinator community at the Soebats site might favour traits that facilitate a broad fundamental niche, where a broad fundamental pollination niche can act as reproductive assurance mechanism against pollinator abundance fluctuations (Waser *et al.* 1996). In the networks sampled in 2015, a tabanid (*Rhigioglossa* sp.) dominated interactions with the soeb morph, but this was not the case during the transplant experiments in 2016, when *Rhigioglossa* sp. emerged in much lower abundances, and interactions were dominated by large bee flies. This is consistent with work in Mediterranean scrub communities showing that interannual fluctuations in pollinator occurrences result in plant species having broad pollination niches when assessed across years, even though they might have narrow realized pollination niches within a flowering season (Petanidou *et al.* 2008). Further, visitation rates at Soebats were generally lower than at Kamies, and pollinator limitation might favour broad, rather than narrow, fundamental pollination niches (Aigner 2001). That is,

the soeb morph might thus have floral traits that consistently attract many pollinator species irrespective of community context. This suggests that different Namaqualand taxa are using different strategies to optimize their fitness.

Our results have important implications for bipartite interaction network studies that typically assume observed interactions reflect fundamental pollination niches. Although in some daisies (e.g. the cal morph) the observed niche is fundamental, for others it might not be (see Table 1). The breadth of the fundamental niche can only be elucidated through experimentation, and through thorough spatial and temporal sampling replication. The calculation of pollination specificity without verifying niche breadths is likely to strongly overestimate how functionally specialized pollination interactions are. In contrast, studies that do not incorporate the evenness of interactions are likely to underestimate ecological specialization, particularly in actinomorphic species that are likely to be visited by many opportunistic species that contribute little to pollination.

Conclusions

Here we show ecological specialization in Namaqualand daisies, challenging the traditional view that actinomorphic flowers have broad pollination niches. Our results suggest that quantitative, rather than qualitative differences in pollinator attraction is prevalent; that is, although plants are generally visited by many pollinator species, some pollinator species are attracted disproportionately more than others. We suggest that narrow fundamental pollination niches can evolve in actinomorphic species without alteration to floral symmetry through selection on visual signal and reward-related traits.

Tables

Table 5.1. Plant pollination niche breadths for all daisy species in six networks sampled in two community types (Kamies and Soebats). Two specialization metrics were calculated: (R) the number of visitor species to a plant species (interaction partner richness), and (D) the number of visitor species to a plant species weighted by interaction frequencies (interaction partner diversity). Z-scores from null models were used to assess whether plant species were visited by fewer (indicated in bold) or more (indicated with an asterisk) interaction partners than expected under random (plain text) visitation. Most plant species had narrower pollination niches than expected from random visitation (richness: 55%; diversity: 68%), and few plant species had broader pollination niches than expected from random (richness: 27%; diversity: 7%). The number of insect species available in each network is indicated in brackets next to the network number.

Community		Kamies						Soebats					
Network (Number of insect species)		1 (40)		2 (36)		3 (40)		1 (23)		2 (20)		3 (28)	
Species	Annual/ Perennial	R	D	R	D	R	D	R	D	R	D	R	D
Arctotheca calendula	A			**7**	**6.02**								
Gorteria diffusa (Cal)	A	**2**	**1.01**	**2**	**1.15**	**3**	**1.08**						
Cotula sp.	A	**7**	**5.01**	**7**	6.26	**1**	**1**	12*	6.52	6	4.65	**2**	**1.66**
Didelta spinosa	P	24*	10.72*	12*	9.38*	13	8.56						
Dimorphotheca sinuata	A	**6**	**1.27**	11	**3.71**	13	**3.38**	12*	4.45			**8**	**2.13**
Felicia australis	A	13*	6.11			12	**6.88**	**3**	**2.55**			**8**	7.07
Ferlicia bergeriana	A			14*	10.09*					6	**2.74**		
cf. Leysera sp.	A	**6**	5.47	**7**	6.54			7	**2.30**	8	**2.99**	**8**	6.28
Senecio cardiminifolius	A			**5**	**4.77**	13	8.05						
Ursinia cakilefolia	A	20*	**2.43**										
Cotula sp.2	P					**4**	**2.38**						
Osteospermum amplectans	A					**8**	**4.90**						
Osteospermum hyoseroides	A					16*	**6.14**						
Arctotis fastuosa	A											**6**	**5.43**
Didelta carnosa	A									10*	**1.78**	**3**	**1.93**
Gazania tenuifolia	A											**1**	**1.00**
Gorteria diffusa (Soeb)	A							13*	**4.07**	13*	**1.78**	17*	**5.08**
Gazania leiopoda (orange)	P									**3**	**1.59**		
Gazania leiopoda (red)	P									**1**	**1.00**	**2**	**1.99**
% Specialist		**57**	**57**	**63**	**40**	**44**	**78**	**20**	**60**	**29**	**86**	**89**	**78**
% Generalist		**43**	**14**	**25**	**25**	**11**	**0**	**60**	**0**	**29**	**0**	**11**	**0**

Table 5.2. Various floral traits are associated with pollination niche breadths. We tested for the association between pollination niche breadths and floral traits (i.e. floral visual signal and reward-related traits) using linear models. We used two pollination niche breadth metrics (interaction partner richness: number of species a plant species interacts with; interaction partner diversity: the number of species a plant species interacts with weighted by their visitation frequency), and we calculated these using insect species and insect functional groups respectively. Significance at $p < 0.05$ is indicated in bold. For dominant flower colour, eight categories were included in the model, and we show in brackets how many of these were significantly associated with our response variables. We also indicate the R-squared and p-values for each of the four linear models.

		Insect species				Insect functional groups			
		Interaction richness		Interaction diversity		Interaction richness		Interaction diversity	
		t	p	t	p	t	p	t	p
Visual signals	Dominant flower colour		**<0.05 (5)**		**<0.05 (1)**		**<0.05 (5)**		NS
	Complexity(1)	**-2.577**	**0.01**	-1.363	0.18	**-2.097**	**0.04**	-0.342	0.74
	Complexity (2)	1.800	0.08	-0.160	0.87	1.012	0.32	-1.019	0.32
	log(total signal size)	**3.273**	**0.002**	**3.107**	**0.004**	**3.529**	**0.001**	**2.278**	**0.03**
Reward traits	log(nectar tube length)	**-2.252**	**0.03**	-1.528	0.14	-1.482	0.15	-0.509	0.61
	log(number of florets+1)	1.648	0.11	1.407	0.17	**2.353**	**0.02**	1.111	0.28
R^2			0.48		0.40		0.45		0.31
p			<0.001		0.003		0.001		0.02

Table 5.3. Results from transplant experiment that tested whether niche breadths vary with community context. Analysis of variance was used to test the effects of *Gorteria diffusa* phenotype (cal versus soeb), heterospecific competition treatment (high versus low), and site (Kamies versus Soebats) on (1) interaction partner richness, (2) interaction partner diversity and (3) visitation rates to *G. diffusa* in experimental arrays. Significance at $p < 0.05$ is indicated in bold.

Effects	Partner richness		Partner diversity		Visitation rate	
	F	p	F	p	F	p
Phenotype	**18.232**	**<0.001**	**19.021**	**<0.001**	2.606	0.12
Competition	0.455	0.51	0.046	0.83	0.113	0.74
Site	0.051	0.82	0.218	0.65	**6.977**	**0.02**

Figures

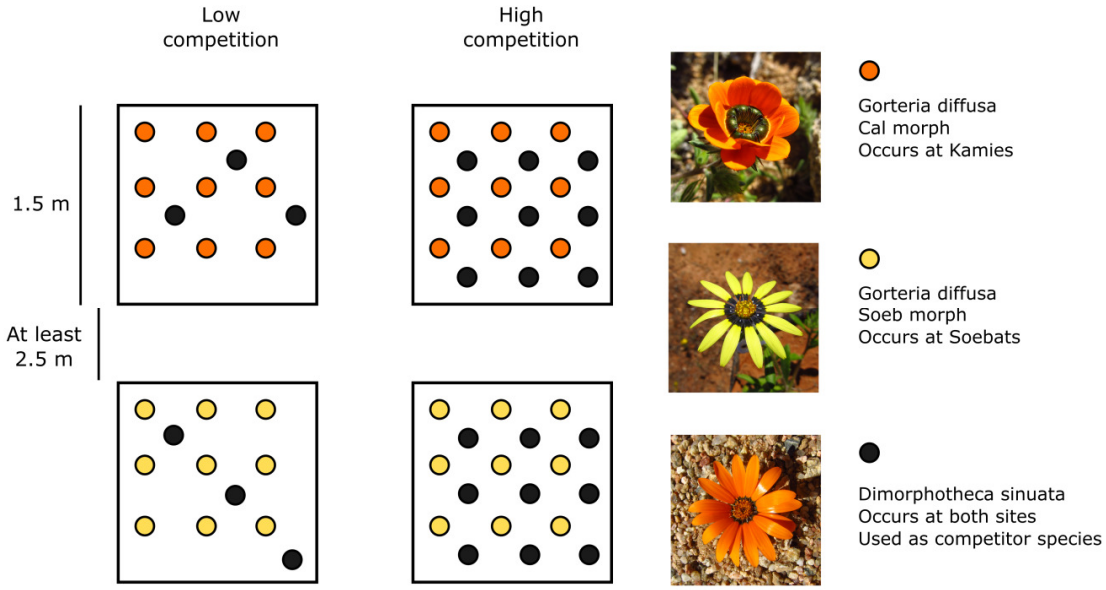

Figure 5.1. The setup of the transplant experiment that tested whether niche breadths vary with community context is shown. The cal and soeb morphs of *Gorteria diffusa* were transplanted in arrays to Kamies and Soebats. Three replicates of the depicted setup were created at each of the two sites, and pollinator visitation rates and visitor identities were recorded. *Dimorphotheca sinuata* was used as competitor species in varying densities and was used, along with the natural variation in pollinator community composition between Kamies and Soebats, to establish whether pollination specialization varies with community context.

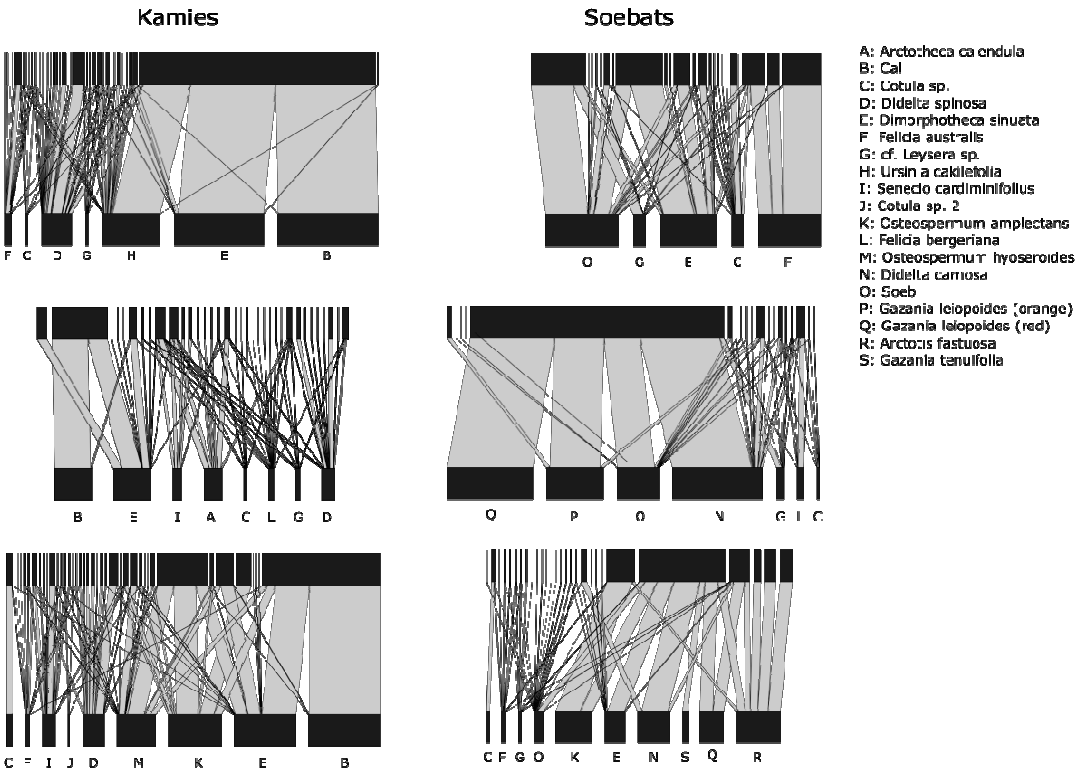

Figure 5.2. The six flower visitor networks sampled are shown. Three networks were respectively sampled for the Kamies and Soebats community types. Interaction strengths (grey bars) are based on the number of visits per flower (per 15 minute interval). Lower black bars with letters show plant species, and upper black bars shown insect species.

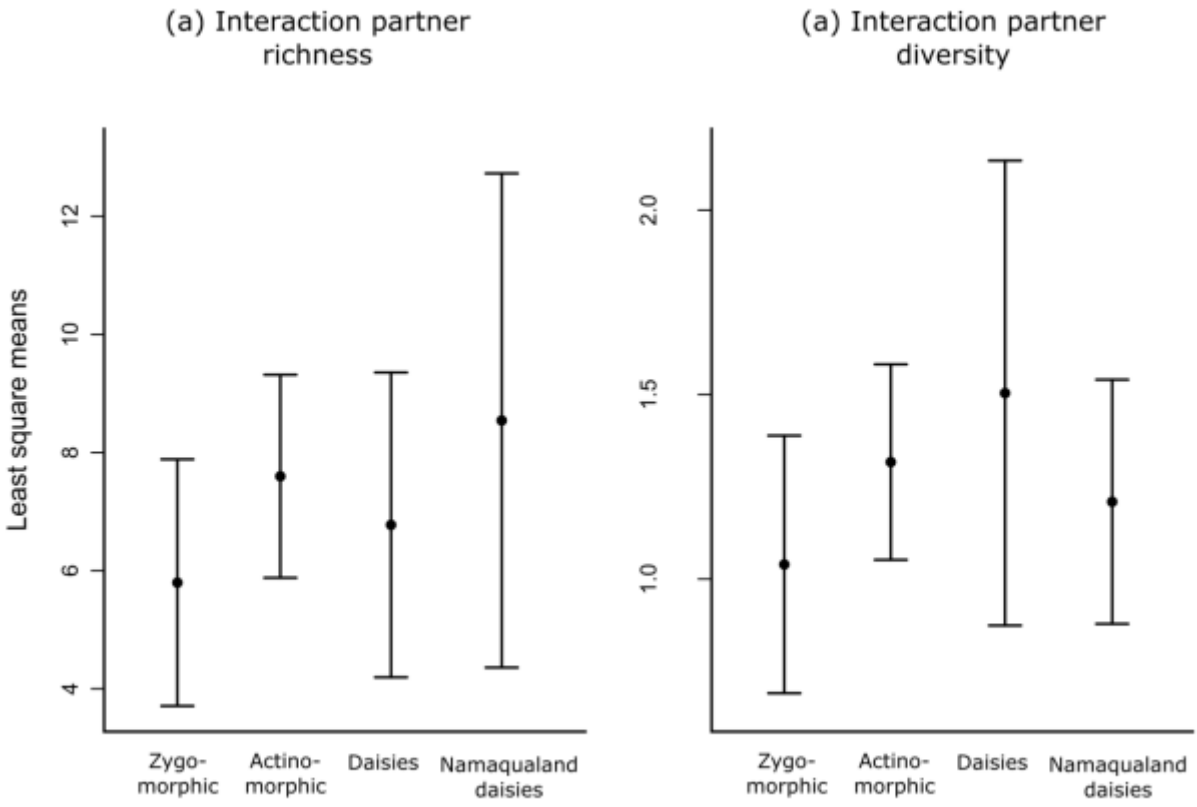

Figure 5.3. We compared pollination niche breadths of Namaqualand daisies to zygomorphic and actinomorphic species from other parts of the world. Zygomorphic species had significantly lower interaction partner richness than all other groups, but groups did not differ in interaction partner diversity. Least square means from the models are plotted (with estimated standard errors), and models controlled for which network the data came from.

Chapter 6

Conclusions

Throughout my thesis I investigated how mutualists and antagonists interact with flower colour. I assessed how these interactions drive community assembly patterns, and how they influence niche breadth evolution.

I found that complex flower colour patterns show clustered community assembly, which suggests that pollinator mediated assembly need not involve competitive interactions. Instead when pollinator communities are dominated by one or few species, the benefits of pollinator sharing might outweigh the costs of competition to generate clustered assembly. This contrasts with the general assumption that competition is important in driving floral evolution.

I show a strong geographic mosaic of dominant pollinator species with largely non-overlapping distribution ranges and divergent innate colour preferences. The non-overlapping ranges of these dominant Bombyliid species potentially results from different abiotic or biotic requirements, such as parasitizing different host insects, and the spatial distribution of these resources thus indirectly impact flower colour distribution in Namaqualand. This geographic mosaic of dominant pollinator species potentially underlies the clustering of flower colours into communities that was shown in chapter 2, with different pollinator species dominating pollination interactions in different communities.

Further, daisies that close for some hours during the day have cryptic lower petal surface colouration that reduces herbivory rates. Reducing the loss of flowers may be particularly important in annual species with long-lived flowers, and cryptic colouration might have fewer trade-offs than chemical defences. Camouflage in plants remains an understudied phenomenon, and my results suggest that crypsis may be an effective defence strategy for floral tissue during times when pollinators are inactive.

Chapter 2, 3 and 4 showed that mutualists and antagonists jointly exert selection on flower colour. In chapter 5, I show that this selection has resulted in narrow and broad evolved fundamental pollination niches, and that different daisy species are optimizing their fitness in different ways. The outcome of selection likely results from the plant and insect community context that different daisy species occur in, where in areas with low pollinator abundances (or temporally variable pollinator communities) it might be beneficial to attract as many pollinator species as possible. In contrast, in areas with many pollinators and florivores, it might be beneficial to exclude costly visitors from visitation. Particularly, I show that plant species with long nectar tubes and complex colour patterns are visited by few pollinator species, and this suggests that some plant species might be reducing their pollination niche by preventing costly visitors from accessing rewards or from exploiting certain foraging behaviours.

Taken together, my research adds to the accumulating evidence that both pollinators and antagonists are important determinants of selection and ecological sorting of flower colour. Particularly, my findings challenge the prevailing perception of the generalised pollination systems of daisies, and suggest that daisy colour represents a pollination syndrome trait. I show that different pollinator species within a functional group (i.e. different bee fly species) exhibit divergent innate colour preferences, which has important implications for studies that create functional groups based on phylogenetic relatedness. My results also suggest that quantitative, rather than qualitative differences in pollinator attraction are prevalent, and studies that do not take interaction evenness into account might be overestimating pollination niche breadths. I show that fundamental pollination niches can evolve in actinomorphic species without alteration to floral symmetry through selection on visual signal and reward-related traits, and my thesis highlights how spatial variation in plant and insect community composition can shape floral trait evolution.

One major obstacle in this thesis was the lack of information on fly visual systems, and on how flower colour and colour patterns influence fly foraging behaviour. The role of flies as pollinators is becoming increasingly clear, particularly in desert systems or areas with high wind speeds, but research on how flies drive floral evolution is still lacking. Because flies potentially require different resources (e.g. they do not collect large pollen volumes) and potentially exhibit different foraging behaviours than bees, we might expect different evolutionary outcomes in communities where flies are the primary pollinators instead of bees. Future work should focus on how pollinating flies (such as Bombyliidae) perceive colour (categorical versus continuous vision), and on how their foraging behaviour is influenced by flower shape, colour and patterning.

Appendix 1

Chapter 2

Phylogeny inference

Initial automated alignment used the ClustalW method in the 'msa' package in R. This was manually checked and corrected for consistency across taxa in BioEdit Sequence Alignment Editor (Hall 1999); the alignment (in BEAST .xml input format) is available on request from the corresponding author. Phylogeny was estimated in the BEAST v. 1.8.3 software (Drummond *et al.* 2012). Substitution models for each gene region (GTR + I + G for the ITS, *matK*, *ndhF* and *rbcL*, and GTR + G for the ETS and *trnL-trnF* regions) were selected using the AIC criterion in MrModeltest (Nylander 2004). Substitution models were unlinked in BEAUti v. 1.8.3 but the same clock and tree model was implemented for all partitions. In order to obtain the correct placement of the root, monophyly was enforced for the tribes in subfamily Asteroideae (all tribes except Arctotideae). An uncorrelated relaxed clock was implemented, with the rate parameter for each branch drawn from a lognormal distribution. Divergence ages were not calibrated to real time, since we were interested only in relative divergence times, and due to the extremely scanty nature of the fossil record in Asteraceae. Due to the very sparse sampling of the large family Asteraceae (> 20,000 species), we implemented a Yule birth-death tree prior with incomplete sampling (Stadler 2009), with the

birthdeath.sampleProbability prior drawn from an exponential distribution having a mean of 0.5 and 95 % confidence interval (CI) between 0.013 and 1.84. The birthDeath.meanGrowthRate prior was drawn from a normal distribution with a mean of 64, standard deviation of 60 and truncated between [0; 600]. The birthDeath.relativeDeathRate prior was also set to a normal distribution, with mean of 0.5, standard deviation of 1.0 and truncated between [0; 10.0]. The clock variation prior ucld.stdev was set to an exponential distribution with a mean of 0.333, giving a 95 % CI between 0.008 and 1.23. Default values were used for all other priors. Four independent MCMC runs, each with a chain length of 10 million, were initiated from independent random starting trees, with parameters logged to file every 1000 steps. Convergence of the chains, and the number of samples to be discarded as burn-in, was assessed by examining likelihood traces and comparing parameter estimates and effective sample sizes (ESS) in Tracer v1.6.0 (Rambaut *et al.* 2014). After discarding the burn-in fraction, the 36,000 trees and parameter samples from all four runs were combined in LogCombiner v1.8.3 (Drummond *et al.* 2012) and the annotated maximum clade credibility (MCC) tree produced using the TreeAnnotator and FigTree software (from the BEAST v. 1.8.3 package; Drummond *et al*. 2012).

Table S2.1. Species occurrences (presence/absence) for the twenty sampled sites. Taxonomy according to Manning & Goldblatt (2012); 'form' refers to different colour morphs of the same species, identified in the present study.

spp	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	Site 15	Site 16	Site 17	Site 18	Site 19	Site 20
Amellus.alternifolius Roth.														X						
Amellus.coilopodius DC.						X	X													
Amellus microglossus DC.			X			X			X						X					
Arctotheca calendula (L.) Levyns		X		X	X				X	X	X	X	X				X			
Arctotis adpressa DC.	X										X	X								
Arctotis cf. acaulis L.		X													X					
Arctotis fastuosa Jacq.					X				X									X		
Arctotis sp.																		X	X	
Berkheya fruticosa (L.) Erhr.										X					X					
Berkheya spinosissima (Thunb.) Willd.				X					X											
Didelta carnosa (L.f.) Aiton, form A			X	X										X						
Didelta carnosa (L.f.) Aiton, form B							X	X	X							X			X	
Didelta spinosa (L.f.) Aiton, form A										X			X	X						
Didelta spinosa (L.f.) Aiton, form B				X					X							X				
Dimophotheca pluvialis (L.) Moench																	X	X	X	X
Dimorphotheca pinnata (Thunb.) Harv., form A			X	X	X	X	X													
Dimorphotheca pinnata (Thunb.) Harv., form B														X	X	X				
Dimorphotheca polyptera DC.															X					
Dimorphotheca sinuata DC., form A	X	X		X			X	X	X		X	X	X							
Dimorphotheca sinuata DC., form B										X				X	X	X				
Dimorphotheca tragus (Aiton) B.Nord.											X				X					
Eriocephalus punctulatus DC.											X									

Euryops multifidus (Thunb.) DC.														X			
Euryops tenuissimus (L.) DC.													X				
Felicia australis (Alston) E.Phillips	X	X		X					X		X	X	X		X		
Felicia bergeriana (Spreng.) O.Hoffm.		X		X		X	X			X		X	X		X		
Felicia cf. dregei DC.										X	X						
Felicia dubia Cass.								X									
Felicia hirsuta DC.															X		
Felicia merxmuelleri Grau						X											
Gazania heterochaeta DC.															X	X	
Gazania krebsiana Less.														X			
Gazania leiopoda (DC.) Roessler		X				X		X		X	X	X					
Gazania lichtensteinii Less.															X		
Gazania tenuifolia Less.			X			X	X	X									
Gorteria diffusa Thunb., Steinkopf form															X		
Gorteria diffusa Thunb., Buffels. form								X									
Gorteria diffusa Thunb., Cal. form		X															
Gorteria diffusa Thunb., Garies form	X			X	X												
Gorteria diffusa Thunb., Okiep form														X		X	
Gorteria diffusa Thunb., Soebats. form						X	X										
Gorteria diffusa Thunb., Spring. form									X								
Gymnodiscus linearifolia DC.										X	X						
Hirpicium alienatum (Thunb.) Druce, form A															X		
Hirpicium alienatum (Thunb.) Druce, form B						X		X									
Hirpicum echinus Less.															X		
Lasiospermum brachyglossum DC.			X		X	X									X		
Leysera gnaphalodes (L.) L.				X					X					X			
Leysera tenella DC.			X		X								X		X	X	
Osteospermum monstrosum (Burm.f.) J.C.Manning & Goldblatt			X				X						X	X			

Osteospermum amplectens (Harv.) Norl., form A	X	X	X	X	X		X		X	X					X	X						
Osteospermum amplectens (Harv.) Norl., form B																				X		
Osteospermum hyoseroides (DC.) Norl.		X			X					X												
Osteospermum microcarpum (Harv.) Norl.			X			X			X													
Osteospermum rigidum Aiton															X							
Othonna cylindrica (Lam.) DC.				X					X													
Othonna macrophylla DC.													X									
Rhynchopsidium pumilum (L.f.) DC.			X	X	X			X	X	X				X	X							
Senecio abruptus Thunb.									X													
Senecio arenarius Thunb.											X		X		X						X	
Senecio cardaminifolius DC.	X	X	X	X	X					X		X			X	X						
Senecio cf. arenarius Thunb., yellow form													X									
Senecio cf. cinerascens Aiton													X									
Senecio cf. erosus L.f. [= *S. eriobasis* DC.]														X		X						
Senecio cinerascens Aiton				X									X									
Senecio sp. shrub				X																		
Osteospermum cf. *scariosum* DC. [=*Tripteris cf.aghillana* DC.]													X			X						
Osteospermum oppositifolium (Aiton) Norl. [=*Tripteris oppositifolia* (Aiton) B.Nord.]				X					X								X					
Osteospermu sinuatum (DC.) Norl. var. *sinuatum* [=*Tripteris sinuata* DC. var. *sinuata*]	X			X					X					X								
Osteospermum sinuatum (DC.) var. *lineare* (Harv.) Norl. [=*Tripteris sinuata* DC. var. *linearis* (Harv.) B.Nord.]															X							
Ursinia anthemoides (L.) Poir. subsp. *anthemoides*				X								X										
Ursinia anthemoides (L.) Poir. subsp. *versicolor* (DC.) Prassler				X																		
Ursinia cakelifolia DC.					X																	
Ursinia calenduliflora (DC.) N.E.Br., short form with shiny band															X	X						
Ursinia calenduliflora (DC.) N.E.Br., tall form									X				X									
Ursinia chrysanthemoides (Less.) Harv.		X									X											

Ursinia kamiesbergensis Magee & Mucina												X	X										
Ursinia nana DC.			X						X						X	X	X						
Ursinia speciosa DC.																			X		X		
Chrysochoma ciliata L.												X	X		X								
Cotula barbata DC.		X										X	X	X									
Cotula coronopifolia L.	X	X	X	X			X		X														
Cotula leptalea DC.									X	X						X							
Cotula microglossa (DC.) O.Hoffm. & Kuntze ex Kuntze	X			X										X									
Foveolina dichotoma (DC.) Källersjö			X		X	X	X	X	X														
Helichrysum hebelepis DC.									X								X						
Oncosiphon grandiflorus (Thunb.) Källersjö							X																
Oncosiphon suffruticosus (L.) Källersjö			X	X		X			X										X				
Othonna cf. intermedia Compton															X	X							
Pteronia glabrata L.f.								X															

Table S2.2. Genbank accession numbers for the sequences of generic representatives used in the phylogenetic reconstruction.

Genus	ITS	rbcL	ndhf	trnL-trn-f	ETS
Amellus	FJ457933.1	AM234845.1	–	–	DQ479052.1
Arctotheca	DQ444720	AM234848	DQ889661	DQ889645.1	–
Arctotis	JN837105	EU384947	EU385133	EU846505	–
Berkheya	EU527195.1	AM234854.1	EU527295.1	EU527245.1	EF556318.1
Didelta	AY504717.1	AM234865.1	AY504759.1	AY504799.1	–
Dimorphotheca	KM356171.1	L13636.1	L39438.1	EU385060.1	AF319696.1
Eriocephalus	EF155771.1	–	AF153645.1	AF452502.1	–
Euryops	EF538209.1	AM234870.1	EF537966.1	AY952926.1	–
Felicia	AY193799.1	L13639.1	L39445.1	EU385068.1	–
Gazania	AY504720.1	L13638.1	L39423.1	AY504802.1	EF556314.1
Gorteria	AY504722.1	AM234875.1	EU385168.1	AY504804.1	JQ220319.1
Gymnodiscus	EF538217.2	AM234876.1	GU817864.1	GU818005.1	GU818173.1
Hirpicium	AY504724.1	–	AY504764.1	DQ444807.1	JQ220301.1
Lasiospermum	AM774459.2	AM234885.1	AF153624.1	–	–
Leysera	KR559464.1	AM234886.1	HM445663.1	AF100473.1	KT865366.1
Osteospermum	AF422131.1	EU385004.1	L39440.1	EU385097.1	AF319733.1
Othonna	AF459960.1	GU817777.1	EF537981.2	EF028727.1	GU818218.1
Rhynchopsidium	KT865592.1	AM234908.1	–	FR822647.1	KT865419.1
Senecio	AF457421.1	L13933.1	L39435.1	GU818078.1	GU818280.1
Tripteris	FJ861471.1	AM234918.1	–	FJ861559.1	–
Ursinia	AF046940.1	EU385026.1	EU385215.1	EU385121.1	–
Chrysocoma	FJ457941.1	AM234859.1	–	–	–
Cotula	AY603260.1	KT626684.1	GU817849.1	GU817960.1	GU818143.1
Foveolina	KP718473.1	AM234871.1	–	–	–
Helichrysum	HE611525.1	GU817766.1	HE612157.1	GU818009.1	GQ913876.1
Oncosiphon	AH011694.2	EU385002.1	EU385189.1	EU385095.1	–
Pteronia	AF046947.1	AM234905.1	–	–	DQ479142.1

Table S2.3. Assignment of species to colour pattern categories using raw spectra*, bee vision, and fly vision

Species	raw spectra	bee	fly
Amellus.alternifolius Roth.	A	C	1
Amellus.coilopodius DC.	B	C	1
Amellus microglossus DC.	A	C	1
Arctotheca calendula (L.) Levyns	C	M	2
Arctotis adpressa DC.	D	D	1
Arctotis cf. acaulis L.	E	E	2
Arctotis fastuosa Jacq.	F	K	3
Arctotis sp.	E	Q	2
Berkheya fruticosa (L.) Erhr.	G	I	1
Berkheya spinosissima (Thunb.) Willd.	H	E	1
Didelta carnosa (L.f.) Aiton, form A	H	G	1
Didelta carnosa (L.f.) Aiton, form B	G	I	1
Didelta spinosa (L.f.) Aiton, form A	I	H	1
Didelta spinosa (L.f.) Aiton, form B	H	H	1
Dimophotheca pluvialis (L.) Moench	J	B	2
Dimorphotheca pinnata (Thunb.) Harv., form A	D	T	1
Dimorphotheca pinnata (Thunb.) Harv., form B	K	S	4
Dimorphotheca polyptera DC.	I	K	5
Dimorphotheca sinuata DC., form A	D	T	4
Dimorphotheca sinuata DC., form B	K	S	6
Dimorphotheca tragus (Aiton) B.Nord.	L	N	2
Eriocephalus punctulatus DC.	A	C	1
Euryops multifidus (Thunb.) DC.	H	G	1
Euryops tenuissimus (L.) DC.	M	K	5
Felicia australis (Alston) E.Phillips	N	C	1
Felicia bergeriana (Spreng.) O.Hoffm.	B	C	1
Felicia cf. dregei DC.	N	C	1
Felicia dubia Cass.	B	C	1
Felicia hirsuta DC.	N	C	1

Felicia merxmuelleri Grau	B	C	1
Gazania heterochaeta DC.	O	R	4
Gazania krebsiana Less.	D	T	1
Gazania leiopoda (DC.) Roessler	P	T	1
Gazania lichtensteinii Less.	G	I	1
Gazania tenuifolia Less.	O	R	5
Gorteria diffusa Thunb., Steinkopf form	Q	R	4
Gorteria diffusa Thunb., Buffels. form	D	T	1
Gorteria diffusa Thunb., Cal. form	D	P	1
Gorteria diffusa Thunb., Garies form	D	T	1
Gorteria diffusa Thunb., Okiep form	P	E	1
Gorteria diffusa Thunb., Soebats. form	Q	J	1
Gorteria diffusa Thunb., Spring. form	P	E	1
Gymnodiscus linearifolia DC.	H	E	1
Hirpicium alienatum (Thunb.) Druce, form A	R	D	5
Hirpicium alienatum (Thunb.) Druce, form B	G	I	1
Hirpicum echinus Less.	G	I	1
Lasiospermum brachyglossum DC.	S	A	1
Leysera gnaphalodes (L.) L.	H	E	1
Leysera tenella DC.	H	E	1
Osteospermum monstrosum (Burm.f.) J.C.Manning & Goldblatt	F	K	7
Osteospermum amplectens (Harv.) Norl., form A	F	L	2
Osteospermum amplectens (Harv.) Norl., form B	T	K	7
Osteospermum hyoseroides (DC.) Norl.	U	L	2
Osteospermum microcarpum (Harv.) Norl.	I	K	5
Osteospermum rigidum Aiton	V	K	5
Othonna cylindrica (Lam.) DC.	I	K	1
Othonna cf. intermedia Compton	I	K	1
Othonna macrophylla DC.	I	K	1
Rhynchopsidium pumilum (L.f.) DC.	H	E	1
Senecio abruptus Thunb.	G	I	1
Senecio arenarius Thunb.	B	C	1
Senecio cardaminifolius DC.	G	I	1
Senecio cf. arenarius Thunb., yellow form	I	O	1
Senecio cf. cinerascens Aiton	G	K	1

Senecio cf. erosus L.f. [= *S. eriobasis* DC.]	W	E	1
Senecio cinerascens Aiton	G	I	1
Senecio sp. shrub	H	D	1
Osteospermum cf. *scariosum* DC. [=*Tripteris cf.aghillana* DC.]	I	K	5
Osteospermum oppositifolium (Aiton) Norl. [=*Tripteris oppositifolia* (Aiton) B.Nord.]	U	L	2
Osteospermu sinuatum (DC.) Norl. var. *sinuatum* [=*Tripteris sinuata* DC. var. *sinuata*]	H	E	1
Osteospermum sinuatum (DC.) var. *lineare* (Harv.) Norl. [=*Tripteris sinuata* DC. var. *linearis* (Harv.) B.Nord.]	H	E	1
Ursinia anthemoides (L.) Poir. subsp. *anthemoides*	X	M	2
Ursinia anthemoides (L.) Poir. subsp. *versicolor* (DC.) Prassler	Z	J	1
Ursinia cakelifolia DC.	AA	L	2
Ursinia calenduliflora (DC.) N.E.Br., short form with shiny band	Q	J	1
Ursinia calenduliflora (DC.) N.E.Br., tall form	O	K	1
Ursinia chrysanthemoides (Less.) Harv.	AB	F	2
Ursinia kamiesbergensis Magee & Mucina	U	L	2
Ursinia nana DC.	G	I	1
Ursinia speciosa DC.	J	B	2
Chrysochoma ciliata L.	Y	Y	8
Cotula barbata DC.	Y	Y	8
Cotula coronopifolia L.	Y	Y	8
Cotula leptalea DC.	Y	Y	8
Cotula microglossa (DC.) O.Hoffm. & Kuntze ex Kuntze	Y	Y	8
Foveolina dichotoma (DC.) Källersjö	Y	Y	8
Helichrysum hebelepis DC.	Y	Y	8
Oncosiphon grandiflorus (Thunb.) Källersjö	Y	Y	8
Oncosiphon suffruticosus (L.) Källersjö	Y	Y	8
Pteronia glabrata L.f.	Y	Y	8

*As readers are generally not familiar with the appearance of the daisies we work on, we describe the different colour pattern categories (based on raw spectra) as humans would see them subjectively. Some of the colours would fall into the same group for humans, but the spectral peaks differed enough to be placed in separate groups (based on inflection points), eg. CPC I and M would seem similar in human vision, but spectral peaks differed enough to be placed in different categories.

A: white (OR), white (IR), yellow (D)
B: purple (OR), purple (IR), yellow (D)
C: UV-yellow (OR), yellow (IR), black (D)
D: orange (OR), black (IR), yellow (D)
E: orange (OR), black (IR), black (D)
F: UV-orange (OR), black (IR), black (D)
G: UV-yellow (OR), yellow (IR), yellow (D)
H: yellow (OR), yellow (IR), yellow (D)
I: UV-yellow (OR), UV-yellow (IR), yellow (D)
J: white (OR), white (IR), black (D)
K: UV-orange (OR), black (IR), yellow (D)
L: UV-orange (OR), UV-orange (IR), black (D)
M: UV-yellow (OR), UV-yellow (IR), yellow (D)
N: blue (OR), blue (IR), yellow (D)
O: UV-orange (OR), black (IR), yellow (D)
P: orange (OR), orange (IR), yellow (D)
Q: UV-yellow (OR), black (IR), yellow (D)
R: yelow (OR), UV-yellow (IR), yellow (D)
S: white (OR), black (IR), yellow (D)
T: UV-orange (OR), UV-orange (IR), black (D)
U: UV-orange (OR), orange (IR), black (D)
V: UV-orange (OR), UV-orange (IR), yellow (D)
W: yellow (OR), yellow (IR), yellow (D)
X: UV-yellow (OR), UV-yellow (IR), black (D)
Y: no OR and IR, yellow D
Z: UV-orange (OR), black (IR), yellow (D)
AA: UV-orange (OR), orange (IR), black (D)
AB: orange (OR), orange (IR), black (D)

Table S2.4. Pagel's lambda and p-values are shown for the different CPCs based on raw spectra. Only 7 of the 28 groups show phylogenetic conservatism. Where lambda could not be computed, we used Blomberg's K.

visual type	group	lambda	p	K
raw	A	0.86	0.001	
raw	B	0.19	0.006	
raw	C	NA	0.08	0.3
raw	D	0.07	0.15	
raw	E	0.34	0.008	
raw	F	0.07	0.42	
raw	G	0.12	0.08	
raw	H	0.59	0.001	
raw	I	0.24	0.04	
raw	J	<0.001	1	
raw	K	0.13	0.07	
raw	L	<0.001	1	
raw	M	0.4	0.63	
raw	N	0.33	0.001	
raw	O	<0.001	1	
raw	P	0.05	0.22	
raw	Q	<0.001	1	
raw	R	<0.001	1	
raw	S	NA	0.06	0.59
raw	T	<0.001	1	
raw	U	0.01	0.83	
raw	V	<0.001	1	
raw	W	<0.001	1	
raw	X	<0.001	1	
raw	Y	NA	0.001	1.6
raw	Z	<0.001	1	
raw	AA	<0.001	1	
raw	AB	<0.001	1	

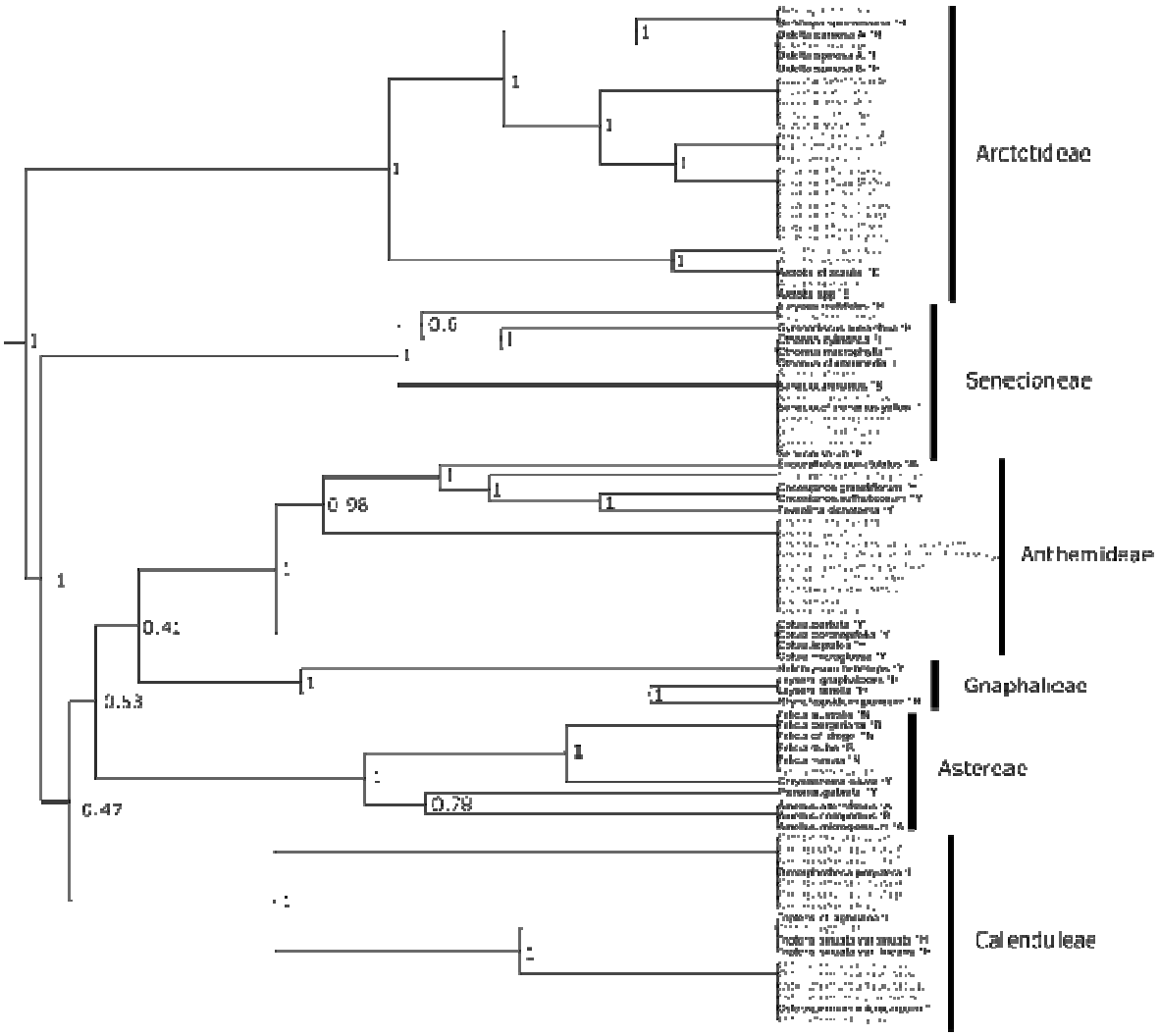

Figure S2.1. BEAST maximum clade credibility tree based on analysis of combined nuclear and plastid DNA. The tree was constructed using genera as terminals; species names have been added in order to indicate the distribution of colour pattern categories. Plant species/morphs that belong to phylogenetically conserved colour pattern categories (based on raw reflectance spectra) are indicated in bold, with the colour pattern group identity indicated

with an asterix (*). Numbers above branches indicate Bayesian posterior probabilities. Bars and names to the right of the taxon names indicate different tribes in the Asteraceae.

Appendix 2

Chapter 5

Table S5.1. Flower visitation networks used to compare pollination niche breadth of Namaqualand daisies to actinomorphic, zygomorphic and non-Namaqualand daisies from other parts of the world. All networks were used to compare interaction partner richness, but only those with visitation rates were used to compare interaction partner diversity.

Number of plant species	Number of pollinator species	Visitation rates recorded? (Yes/No)	Study
87	98	N	Arroyo et al 1982
43	62	N	Arroyo et al 1982
41	28	N	Arroyo et al 1982
32	81	Y	Bartomeus et al 2008
13	13	Y	Bezerra et al 2009
96	276	N	Clements & Long 1923
11	38	N	Dupont et al 2003
23	118	Y	Elberling & Oleson 1999
29	86	N	Hocking 1968
42	91	N	Inouye & Pyke 1988
93	679	N	Kato et al 1990
32	115	N	Kevan 1970
106	54	N	Mcmullen 1993
21	45	N	Medan et al 2002
11	18	N	Mosquin & Martin 1967
13	44	Y	Motten 1982
14	13	Y	Olesen et al 2002
10	12	Y	Olesen et al 2002
33	53	N	Ramirez & Brito 1992
51	25	N	Santos et al 2010
7	32	Y	Schemske et al 1978
13	34	N	Small 1976
15	129	Y	Vazquez & Simberloff 2002

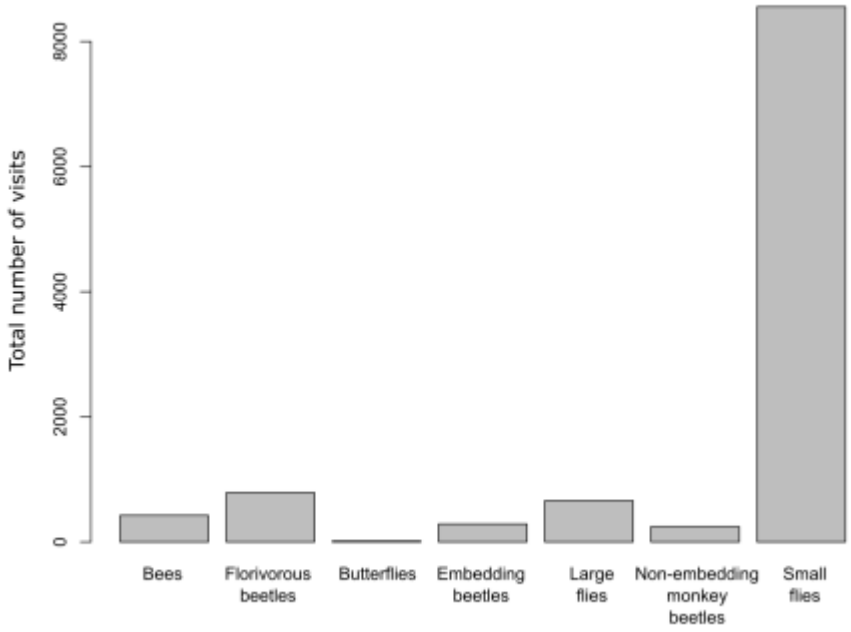

Figure S5.1. The total number of visits by each insect functional group is shown. Interactions are dominated by small flies, and few bees or butterfly visits were recorded.

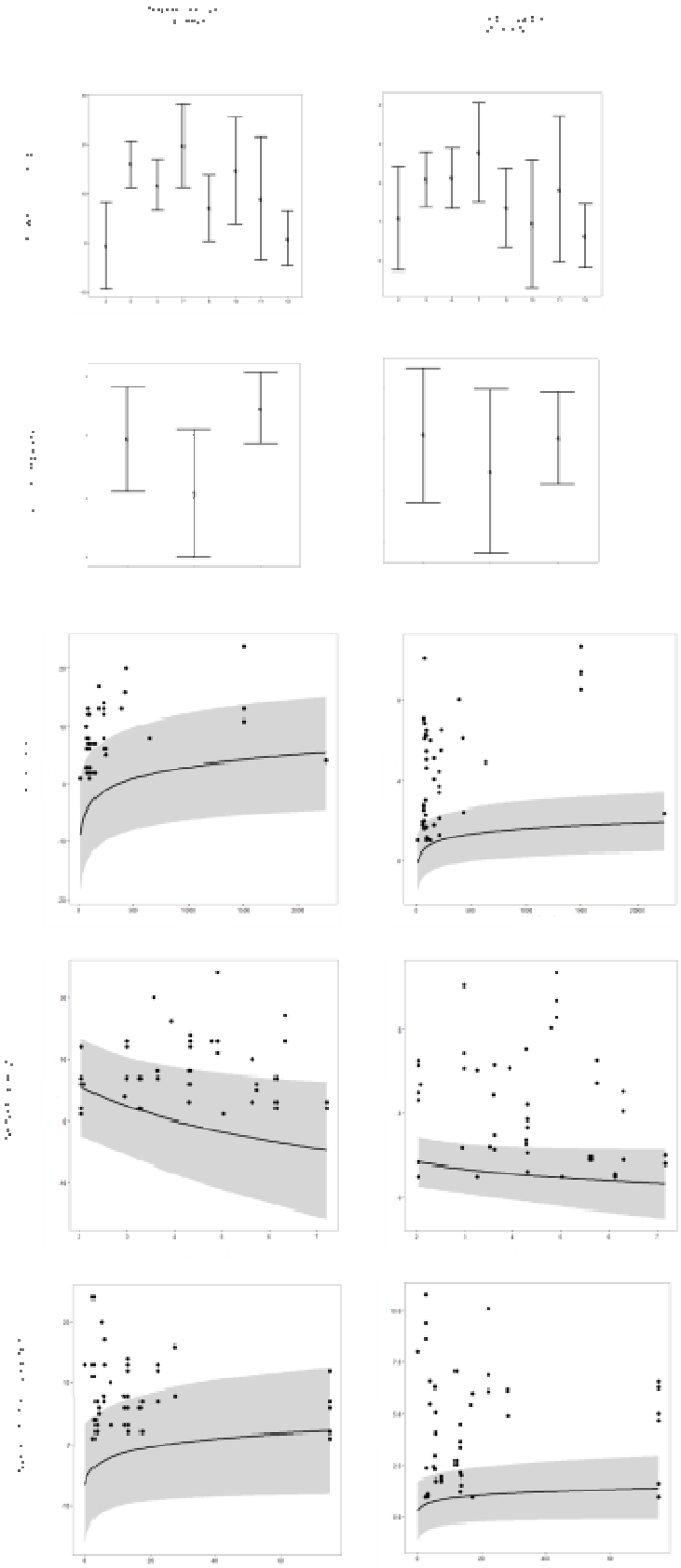

Figure S5.2. We used linear models to test whether interaction partner richness and diversity of daisies were predicted by various visual signal and reward-related traits, and here we plot the residuals of the models.

Bibliography

Literature cited

Adler LS. **2001**. The ecological significance of toxic nectar. *Oecologia* **3**: 409–420.

Adler LS, Seifert MG, Wink M, Morse GE. **2012**. Reliance on pollinators predicts defensive chemistry across tobacco species. *Ecology Letters* **15**: 1140–8.

Aigner PA. **2001**. Optimality modeling and fitness trade-offs: when should plants become pollinator specialists? *Oikos* **95**: 177–184.

Aigner PA. **2004**. Floral specialization without trade-offs: Optimal corolla flare in contrasting pollination environments. *Ecology* **85**: 2560–2569.

Aizen MA, Vazquez DP. **2006**. Flowering phenologies of hummingbird plants from the temperate forest of southern South America: is there evidence of competitive displacement? *Ecography* **29**: 357–366.

Alexandersson R, Johnson SD. **2002**. Pollinator-mediated selection on flower-tube length in a hawkmoth-pollinated *Gladiolus* (Iridaceae). *Proceedings of the Royal Society B* **269**: 631–636.

Anderson B, Johnson SD. **2008**. The geographical mosaic of coevolution in a plant-pollinator mutualism. *Evolution* **62**: 220–225.

Anderson B, Ros P, Wiese TJ, Ellis AG. **2014**. Intraspecific divergence and convergence of floral tube length in specialized pollination interactions. *Proceedings of the Royal Society B:*

Biological Sciences **281**: 20141420.

Arceo-Gómez G, Abdala-Roberts L, Jankowiak A, Kohler C, Meindl GA, Navarro-Fern CM. **2016**. Patterns of among- and within-species variation in heterospecific pollen receipt: The importance of ecological generalization. *American Journal of Botany* **103**: 396–407.

Archibald JK, Wolfe AD, Johnson SD. **2004**. Hybridization and gene flow between a day- and night-flowering species of *Zaluzianskya* (Scrophulariaceae, tribe Manuleeae). *American Journal of Botany* **91**: 1333–1344.

Armbruster WS. **1986**. Reproductive interactions between sympatric *Dalechampia* species: are natural assemblages "random" or organized? *Ecology* **67**: 522–533.

Armbruster WS. **2017**. The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation. *Functional Ecology* **31**: 88–100.

Armbruster WS, Hansen TF, Pélabon C, Pérez-Barrales R, Maad J. **2009**. The adaptive accuracy of flowers: Measurement and microevolutionary patterns. *Annals of Botany* **103**: 1529–1545.

Ashton PS, Givnish TJ, Appanah S. **1988**. Staggered flowering in the Dipterocarpaceae: new insights into floral induction and the evolution of mast fruiting in the aseasonal tropics. *The American Naturalist* **132**: 44–66.

Augustyn WJ, Anderson B, Ellis AG. **2016**. Experimental evidence for fundamental, and not realised, niche partitioning in a plant-herbivore community interaction network. *Journal of Animal Ecology* **85**: 994–1003.

Avarguès-Weber A, Giurfa M. 2014. Cognitive components of color vision in honey bees: How conditioning variables modulate color learning and discrimination. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **200**: 449–461.

Barb JG, Werner DJ, Griesbach RJ. 2008. Genetics and biochemistry of flower colour in stokes aster. *Journal of the American Society for Horticultural Science* **133(4)**: 569-578.

Barbour HR, Archer MA, Hart NS, *et al.* 2002. Retinal characteristics of the ornate dragon lizard, Ctenophorus ornatus. *Journal of Comparative Neurology* **450**: 334–344.

Bascompte J, Jordano P, Melian CJ, Olesen JM. 2003. The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences* **100**: 9383–9387.

Bergamo PJ, Rech AR, Brito VLG, Sazima M. 2016. Flower colour and visitation rates of *Costus arabicus* support the 'bee avoidance' hypothesis for red-reflecting hummingbird-pollinated flowers. *Functional Ecology* **30**: 710–720.

Blanche KR, Ludwig JA, Cunningham SA. 2006. Proximity to rainforest enhances pollination and fruit set in orchards. *Journal of Applied Ecology* **43**: 1182–1187.

Born J, Linder HP, Desmet P. 2007. The Greater Cape Floristic Region. *Journal of Biogeography* **34**:147-162.

Bradshaw HD, Schemske DW. 2003. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**: 176–178.

Braunschmid H, Mükisch B, Rupp T, *et al.* 2017. Interpopulation variation in pollinators

and floral scent of the lady's-slipper orchid *Cypripedium calceolus* L. *Arthropod-Plant Interactions* **11**: 363–379.

Brown BJ, Mitchell RJ, Graham SA. **2002**. Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology* **83**: 2328–2336.

Bukovac Z, Dorin A, Finke V, *et al.* 2017a. Assessing the ecological significance of bee visual detection and colour discrimination on the evolution of flower colours. *Evolutionary Ecology* **31**: 153–172.

Bukovac Z, Shrestha M, Garcia JE, Burd M, Dorin A, Dyer AG. **2017b**. Why background colour matters to bees and flowers. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **203**: 369–380.

Cambell DR, Motten AF. **1985**. The mechanism of competition for pollination between two forest herbs. *Ecology* **66**: 554–563.

Campbell DR, Bischof M, Load JM, Robertson AW. **2010**. Flower color influence insect visitation in alpine New Zealand. *Ecology* **91**: 2638–2649.

Campbell DR, Bischoff M, Lord JM, Robertson AW. **2012**. Where have all the blue flowers gone: Pollinator responses and selection on flower colour in New Zealand *Wahlenbergia albomarginata*. *Journal of Evolutionary Biology* **25**: 352–364.

Cardel YJ, Koptur S. **2010**. Effects of florivory on the pollination of flowers: an experimental field study with a perennial plant. *International Journal of Plant Sciences* **171**: 283–292.

Carlson JE, Holsinger KE, Carlson JE. **2015**. Extrapolating from local ecological processes to genus-wide patterns in colour polymorphism in South African *Protea*.

Proceedings of the Royal Society B **282**: 20150583.

Carroll J, Murphy CJ, Neitz M, Hoeve JN, Neitz J. **2001**. Photopigment basis for dichromatic color vision in the horse. *Journal of vision* **1**: 80–87.

Chamberlain SA, Cartar R V, Worley AC, *et al.* 2014. Traits and phylogenetic history contribute to network structure across Canadian plant–pollinator communities. *Oecologia* **176**: 545–556.

Chittka L. **1992**. The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A* **170**: 533–543.

Chittka L. 1999. Sensorimotor learning in bumblebees: long-term retention and reversal training. *Journal of Experimental Biology* **210**:515-524.

Cooley AM, Carvallo G, Willis JH. **2008**. Is floral diversification associated with pollinator divergence? Flower shape, flower colour and pollinator preference in Chilean *mimulus*. *Annals of Botany* **101**: 641–650.

Coyne JA, Orr HA. **1989**. Patterns of speciation in *Drosophila*. *Evolution* **43**: 362–381.

Daumer K. 1963. Kontrastempfindlichkeit der Bienen für „weiß “verschiedenen UV-Gehalts. *Zeitschrift für vergleichende Physiologie*, **46**(3): 336-350.

De Jager ML, Dreyer LL, Ellis AG. **2011**. Do pollinators influence the assembly of flower colours within plant communities? *Oecologia* **166**: 543–553.

De Jager ML, Ellis AG. **2012**. Gender-specific pollinator preference for floral traits. *Functional Ecology* **26**: 1197–1204.

De Jager ML, Ellis AG. **2013**. The influence of pollinator phylogeography and mate preference on floral divergence in a sexually deceptive daisy. *Evolution* **67**: 1706–1714.

De Jager ML, Ellis AG. **2014**. Costs of deception and learned resistance in deceptive interactions. *Proceedings of the Royal Society B: Biological Sciences* **281**: 20132681.

De Jager ML, Ellis AG. **2014**. Floral polymorphism and the fitness implications of attracting pollinating and florivorous insects. *Annals of Botany* **113**: 213–222.

De Jager ML, Willis-Jones E, Critchley S, Glover BJ. **2017**. The impact of floral spot and ring markings on pollinator foraging dynamics. *Evolutionary Ecology* **31**: 193–204.

De Jong TJ, Klinkhamer PGL, Van Staalduinen MJ. **1992**. The consequences of pollination biology for selection of mass or extended blooming. *Functional Ecology* **6**: 606–615.

De Waal C, Barrett SCH, Anderson B. **2012**. The effect of mammalian herbivory on inflorescence architecture in ornithophilous *Babiana* (Iridaceae): implications for the evolution of a bird perch. *American Journal of Botany* **99**: 1096–103.

De Waal C, Anderson B, Ellis AG. **2015**. Relative density and dispersion pattern of two southern African Asteraceae affect fecundity through heterospecific interference and mate availability, not pollinator visitation rate. *Journal of Ecology* **103**: 513–525.

De Waal C, Rodger JG, Anderson B, Ellis AG. **2014**. Selfing ability and dispersal are positively related, but not affected by range position: A multispecies study on southern African Asteraceae. *Journal of Evolutionary Biology* **27**: 950–959.

Desmet P. 1997. The vegetation and restoration potential of the coastal belt between Port

Nolloth and Alexander Bay, Namaqualand, South Africa. Botany. University of Cape Town, Cape Town.

Desmet PG. **2007**. Namaqualand - A brief overview of the physical and floristic environment. *Journal of Arid Environments* **70**: 570–587.

Dinkel T, Lunau K. **2001**. How drone flies (*Eristalis tenax* L., Syrphidae, Diptera) use floral guides to locate food sources. *Journal of Insect Physiology* **47**: 1111–1118.

Dormann CF, Strauss R. **2014**. A method for detecting modules in quantitative bipartite networks. *Methods in Ecology and Evolution* **5**: 90–98.

Du Plessis M, Johnson SD, Nicolson SW, Bruyns P V., Shuttleworth A. **2018**. Pollination of the "carrion flowers" of an African stapeliad (*Ceropegia mixta*: Apocynaceae): the importance of visual and scent traits for the attraction of flies. *Plant Systematics and Evolution* **304**: 357–372.

Driver A, Desmet PG, Rouget M, Cowling RM, Maze KE. 2003. Succulent karoo ecosystem plan biodiversity component technical report. Cape Conservation Unit, Botanical Society of South Africa Cape Town.

Drummond AJ, Suchard MA, Xie D, Rambaut A. **2012**. Bayensian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.

Dyer AG, Boyd-Gerny S, Shrestha M, *et al.* 2016. Innate colour preferences of the Australian native stingless bee *Tetragonula carbonaria* Sm. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **202**: 603–613.

Dyer AG, Chittka L. 2004. Biological significance of distringuishing between similar

colours in spectrally variable illumination: bumblebees (*Bombus terrestris*): a case study. *Journal of Comparative Physiology A* **190 (2)**:105-114.

Ellis AG, Johnson SD. **2009**. The evolution of floral variation without pollinator shifts in *Gorteria diffusa* (Asteraceae). *American Journal of Botany* **96**: 793–801.

Ellis AG, Johnson SD. **2010**. Floral mimicry enhances pollen export: the evolution of pollination by sexual deceit outside of the orchidaceae. *The American Naturalist* **176**: E143-51.

Ellis AG, Johnson SD. **2012**. Lack of floral constancy by bee fly pollinators: Implications for ethological isolation in an African daisy. *Behavioral Ecology* **23**: 729–734.

Ellis TJ, Field DL. **2016**. Repeated gains in yellow and anthocyanin pigmentation in flower colour transitions in the Antirrhineae. *Annals of Botany* **117**: 1133–1140.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. **2004**. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* **35**: 375–403.

Fishman L, Wyatt R. **1999**. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution* **53**: 1723.

Fleishman LJ, Perez CW, Yeo AI, Cummings KJ, Dick S, Almonte E. **2016**. Perceptual distance between colored stimuli in the lizard *Anolis sagrei*: comparing visual system models to empirical results. *Behavioral Ecology and Sociobiology* **70**: 541–555.

Forest F, Goldblatt P, Manning JC, *et al.* 2014. Pollinator shifts as triggers of speciation in

painted petal irises (*Lapeirousia*: Iridaceae). *Annals of Botany* **113**.

Fukushi T. 1994. Colour perception of single and mixed monochromatic lights in the blowfly *Lucilia cuprina*. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **175**:15–22.

Galen C, Kaczorowski R, Todd SL, Geib J, Raguso RA. **2011**. Dosage-dependent impacts of a floral volatile compound on pollinators, larcenists, and the potential for floral evolution in the Alpine Skypilot *Polemonium viscosum*. *The American Naturalist* **177**: 258–272.

Garcia JE, Spaethe J, Dyer AG. **2017**. The path to colour discrimination is S-shaped: behaviour determines the interpretation of colour models. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **203**: 983–997.

Gegear RJ, Laverty TM. **2005**. Flower constancy in bumblebees: A test of the trait variability hypothesis. *Animal Behaviour* **69**: 939–949.

Geisbauer G, Griebel U, Schmid A, Timney B. **2004**. Brightness discrimination and neutral point testing in the horse. *Canadian Journal of Zoology* **82**: 660–670.

Ghazoul J. **2006**. Floral diversity and the facilitation of pollination. *Journal of Ecology* **94**: 295–304.

Giurfa M, Núñez J, Chittka L, Menzel R. **1995**. Colour preferences of flower-naive honeybees. *Journal of Comparative Physiology A* **177**: 247–259.

Giurfa M, Vorobyev M, Brandt R, Posner B, Menzel R. **1997**. Discrimination of coloured stimuli by honeybees: Alternative use of achromatic and chromatic signals. *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology* **180**: 235–243.

Giurfa M, Vorobyev M, Kevan P, Menzel R. **1996**. Detection of coloured stimuli by honeybees: Minimum visual angles and receptor specific contrasts. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology* **178**: 699–709.

Gómez JM. **2003**. Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *The American Naturalist* **162**: 242–256.

Gómez JM, Perfectti F, Camacho JPM. **2006**. Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *The American Naturalist* **168**: 531–545.

Gong YB, Huang SQ. **2009**. Floral symmetry: Pollinator-mediated stabilizing selection on flower size in bilateral species. *Proceedings of the Royal Society B: Biological Sciences* **276**: 4013–4020.

Goodale E, Kim E, Nabors A, Henrichon S, Nieh JC. **2014**. The innate responses of bumble bees to flower patterns: separating the nectar guide from the nectary changes bee movements and search time. *Naturwissenschaften* **101**: 523–526.

Gotelli NJ. **2000**. Null model analysis of species co-occurrence patterns. *Ecology* **81**: 2606–2621.

Grant V, Grant KA. **1965**. Pollination Ecology. *Science* **150**: 872–873.

Gray M, Stansberry MJ, Lynn JS, Williams CF, White TE, Whitney KD. **2018**. Consistent shifts in pollinator-relevant floral coloration along Rocky Mountain elevation gradients. *Journal of Ecology*: 1–15.

Grossenbacher DL, Stanton ML. **2014**. Pollinator-mediated competition influences selection for flower-color displacement in sympatric monkeyflowers. *American Journal of Botany* **101**: 1915–1924.

Halekoh U, Højsgaard S, Yan J. **2006**. The R Package geepack for generalized estimating equations. *Journal of Statistical Software* **15**: 1–11.

Hansen DM, Van der Niet T, Johnson SD. **2012**. Floral signposts: testing the significance of visual "nectar guides" for pollinator behaviour and plant fitness. *Proceedings of the Royal Society B: Biological Sciences* **279**: 634–639.

Harder LD, Johnson SD. **2009**. Darwin' s beautiful contrivances: evolutionary and functional evidence. *New Phytologist* **183**: 530–545.

Hart NS, Hunt DM. **2007**. Avian visual pigments: characteristics, spectral tuning, and evolution. **169**.

Heystek A, Pauw A. **2014**. Does competition for pollinators contribute to structuring *Erica* communities? *Journal of Vegetation Science* **25**.

Hill PSM, Wells PH, Wells H. **1997**. Spontaneous flower constancy and learning in honey bees as a function of colour. *Animal Behaviour* **54**: 615–627.

Hoballah ME, Gubitz T, Stuurman J, Broger L, Barone M, Mandel T, Dell'Olivo A, Arnold M, Kuhlemeier C. 2007. Singel gene-mediated shift in pollunator attraction in *Petunia*. *The Plant Cell* **19(3)**: 779-790.

Horth L, Campbell L, Bray R. **2014**. Wild bees preferentially visit *Rudbeckia* flower heads with exaggerated ultraviolet absorbing floral guides. *Biology Open* **3**: 221–230.

Irwin RE, Adler LS. **2006**. Correlations among traits associated with herbivore resistance and pollination: implications for pollination and nectar robbing in a distylous plant. *American Journal of Botany* **93**: 64–72.

Jacobs GH. 1981. Comparative color vision. Academic Press, New York.

Jacobs GH. 1993. The distribution and nature of colour vision among the mammals. *Biological Reviews* **68**: 413-471.

Jacobs GH, Deegan II JF, Neitz J. **1998**. Photopigment basis for dichromatic color vision in cows, goats, and sheep. *Visual Neuroscience* **15**: 581–584.

Johnson SD. **1995**. Moth pollination of the cryptic Cape orchid *Monadenia ophrydea*. *Flora* **190**: 105–108.

Johnson SD. **2010**. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **365**: 499–516.

Johnson SD, Dafni A. **1998**. Response of bee-flies to the shape and pattern of model flowers: implications for floral evolution in a Mediterranean herb. *Functional Ecology* **12**: 289–297.

Johnson SD, Linder HP, Steiner KE. **1998**. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany* **85**: 402–411.

Johnson SD, Pauw A, Midgley JJ. **2001**. Rodent pollination in the African lily *Massonia depressa* (Hyancinthaceae). *American Journal of Botany* **88**: 1768–1773.

Johnson SD, Hargreaves AL, Brown M. **2006**. Dark, bitter-tasting nectar functions as a

filter of flower visitors in a bird-pollinated plant. *Ecology* **87**: 2709–2716.

Johnson SD, Ellis AG, Dotterl S. **2007**. Specialization for pollination by beetles and wasps: the role of lollipop hairs and fragrance in *Satyrium microrrhynchum* (Orchidaceae). *American Journal of Botany* **94**: 47–55.

Johnson SD. **2010**. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **365**: 499–516.

Jost L. **2007**. Partitioning diversity into independent alpha and beta components. *Ecology* **88**: 2427–2439.

Junker RR, Höcherl N, Blüthgen N. **2010**. Responses to olfactory signals reflect network structure of flower-visitor interactions. *The Journal of animal ecology* **79**: 818–823.

Junker RR, Blüthgen N, Brehm T, *et al.* 2013. Specialization on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks (T-L Ashman, Ed.). *Functional Ecology* **27**: 329–341.

Kantsa A, Raguso RA, Dyer AG, Sgardelis SP, Olesen JM, Petanidou T. **2017**. Community-wide integration of floral colour and scent in a Mediterranean scrubland. *Nature Ecology and Evolution* **1**: 1502–1510.

Kay KM, Sargent RD. **2009**. The role of animal pollination in plant speciation: integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution, and Systematics* **40**: 637–656.

Keck F. **2015**. phylosignal: Exploring the Phylogenetic Signal in Continuous Traits. R

package version 1.1.

Kembel SW, Cowan PD, Helmus WK, *et al.* 2010. Picante: R tools for integrating phylogenies and ecology.

Kemp JE, Bergh NG, Soares M, Ellis AG. **In press**. Dominant pollinators drive non-random community assembly and shared flower colour patterns in daisy communities. *Annals of Botany*. Available: https://doi.org/10.1093/aob/mcy126

Kessler D, Diezel C, Baldwin IT. **2010**. Changing pollinators as a means of escaping herbivores. *Current Biology* **20**: 237–242.

Kessler D, Diezel C, Clark DG, Colquhoun TA, Baldwin IT. **2013**. *Petunia* flowers solve the defence/apparency dilemma of pollinator attraction by deploying complex floral blends. *Ecology Letters* **16**: 299–306.

Koski MH, Ashman TL. **2014**. Dissecting pollinator responses to a ubiquitous ultraviolet floral pattern in the wild. *Functional Ecology* **28**: 868–877.

Koski MH, Ashman TL. **2015**. An altitudinal cline in UV floral pattern corresponds with a behavioral change of a generalist pollinator assemblage. *Ecology* **96**: 3343–3353.

Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM. **2015**. Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology* **29**: 592–599.

Leonard AS, Brent J, Papaj DR, Dornhaus A. **2013**. Floral nectar guide patterns discourage nectar robbing by bumble bees. *PloS one* **8**: e55914.

Lev-Yadun S. 2014. Defensive masquerade by plants. *Biological Journal of the Linnean*

Society **113**: 1162-1166.

Linder HP. **2003**. The radiation of the Cape flora, southern Africa. *Biological reviews of the Cambridge Philosophical Society* **78**: 597–638.

Liu L, Zhang C, Ji X, Zhang Z, Wang R. **2017**. Temporal petal closure benefits reproductive development of *Magnolia denudata* (Magnoliaceae) in early spring. *Frontiers in Plant Science* **8**: 1–8.

Lunau K. **2014**. Visual ecology of flies with particular reference to colour vision and colour preferences. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **200**: 497–512.

Lunau K, Wacht S. **1994**. Optical releasers of the innate proboscis extension in the hoverfly *Eristalis tenax* L. (Syrphidae, Diptera). *Journal of Comparative Physiology A* **174**: 575–579.

Lunau K, Knüttel H. **1995**. Vision through colored eyes. *Naturwissenschaften* **82**: 432–434.

Lunau K, Papiorek S, Eltz T, Sazima M. **2011**. Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *The Journal of Experimental Biology* **214**: 1607–1612.

Lunau K, Donda M, Hohmann M, Sermon L, Stegmanns V. **2018**. Limitations of learning in the proboscis reflex of the flower visiting syrphid fly *Eristalis tenax*. *PLoS ONE* **13**: 1–20.

MacArthur RH. **1957**. On the realtive abundance of bird species. *Proceedings of the National Academy of Sciences* **43**: 293–295.

Macior LW. **1986**. floral resource sharing by bumblebees and hummingbirds in *Pedicularis* (Scrophulariaceae) pollination. *Bulletin of the Torrey Botanical Club* **113**: 101–109.

Magee AR, Boatwright JS, Mucina L. **2014**. Four new species of *Ursinia* (Asteraceae, Anthemideae) from South Africa, with an updated key to the genus in Namaqualand. *Phytotaxa* **177**: 137–145.

Maia R, Eliason CM, Bitton P-P, Doucet SM, Shawkey MD. **2016**. pavo: an R package for the analysis, visualization and organization of spectral data.

Manning JC, Goldblatt P. **1997**. The *Moegistorhynchus longirostrus* (Diptera: Nemestrinidae) pollination guild: long-tubed flowers and a specialized long-proboscid fly pollination sytem in souther Africa. *Plant Systematics and Evolution* **206**:51-69.

Matsumoto T, Takakura KI, Nishida T. **2010**. Alien pollen grains interfere with the reproductive success of native congener. *Biological Invasions* **12**: 1617–1626.

McCall AC, Irwin RE. **2006**. Florivory: the intersection of pollination and herbivory. *Ecology Letters* **9**: 1351–1365.

McEwen JR, Vamosi JC. **2010**. Floral colour versus phylogeny in structuring subalpine flowering communities. *Proceedings of the Royal Society of London B: Biological Sciences*.

Menzel R. 1967. Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*). *Zeitschrift für vergleichende Physiologie* **56**(1): 22-62.

Milton SJ. **1992**. Effects of rainfall, competition and grazing on flowering of *Osteospermum sinuatum* (Asteraceae) in arid Karoo rangeland. *Journal of the Grassland Society of Southern Africa* **9**: 158–164.

Mitchell RJ, Flanagan RJ, Brown BJ, Waser NM, Karron JD. **2009**. New frontiers in competition for pollination. *Annals of Botany* **103**: 1403–1413.

Mochizuki K, Kawakita A. **2018**. Pollination by fungus gnats and associated floral characteristics in five families of the Japanese flora. *Annals of Botany* **121**: 651–663.

Moeller DA. **2004**. Facilitative interactions among plants via shared pollinators. *Ecology* **85**: 3289–3301.

Morales CL, Traveset A. **2008**. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences* **27**: 221–238.

Muchhala N, Potts MD. **2007**. Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanism, process and pattern. *Proceedings of the The Royal Society B* **274**: 2731–2737.

Muchhala N, Johnsen S, Smith SD. **2014**. Competition for hummingbird pollination shapes flower color variation in Andean Solanaceae. *Evolution* **68**: 2275–2286.

Neal PR, Dafni A, Giurfa M. **1998**. Floral symmetry and its role in plant-pollinator systems: terminology, distribution, and hypotheses. *Annual Review of Ecology and Systematics* **29**: 345–373.

Newman E, Anderson B, Johnson SD. **2012**. Flower colour adaptation in a mimetic orchid. *Proceedings of the Royal Society B* **279**: 2309–2313.

Newman E, Manning J, Anderson B. **2014**. Matching floral and pollinator traits through guild convergence and pollinator ecotype formation. *Annals of Botany* **113**: 373–384.

Newman E, Manning J, Anderson B. **2015**. Local adaptation: Mechanical fit between floral ecotypes of *Nerine humilis* (Amaryllidaceae) and pollinator communities. *Evolution* **69**: 2262–2275.

Ng J, Smith SD. 2016. Widespread flower colour convergence in Solanaceae via alternate biochemical pathways. *New Phytologist* **209(1)**: 407-417.

Nilsson LA. **1988**. The evolution of flowers with deep corolla tubes. *Nature* **334**: 147–149.

Niu Y, Chen G, Peng DL, *et al.* 2014. Grey leaves in an alpine plant: A cryptic colouration to avoid attack? *New Phytologist* **203**: 953–963.

Niu Y, Sun H, Stevens M. **In press.** Plant camouflage: ecology, evolution, and implications. *Trends in Ecology & Evolution*.

Odell E, Raguso RA, Jones KN. **1995**. Bumblebee foraging responses to variation in floral scent and color in Snapdragons (Antirrhinum: Scrophulariaceae). *The American Midland Naturalist* **142**: 257–265.

Oksanen J, Blachett FG, Kindt R, *et al.* 2016. vegan: Community Ecology Package. R package version 2.0-10.

Ollerton J, Killick A, Lamborn E, Watts S, Whiston M. **2007**. Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon* **56**: 717–728.

Ollerton J, Winfree R, Tarrant S. **2011**. How many flowering plants are pollinated by animals? *Oikos* **120**: 321–326.

Olsson P, Lind O, Kelber A. **2017**. Chromatic and achromatic vision: parameter choice and limitations for reliable model predictions. *Behavioral Ecology* **29**: 273-282.

Orford KA, Vaughan IP, Memmott J. **2015**. The forgotten flies: The importance of non-syrphid Diptera as pollinators. *Proceedings of the Royal Society B: Biological Sciences* **282**.

Osorio D, Vorobyev M. 2005. Photoreceptor spectral sensitivities in terrestial animals:

adaptation for luminance and colour vision. *Proceedings of the Royal Society B: Biological Sciences* **272**: 1745-1752.

Ostler WK, Harper KT. **1978**. Floral ecology in relation to plant species diversity in the Wasatch mountains of Utah and Idaho. *Ecology* **59**: 848–861.

Pagel M. **1999**. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

Papiorek S, Rohde K, Lunau K. **2013**. Bees ' subtle colour preferences: how bees respond to small changes in pigment concentration. *Naturwissenschaften*: 633–643.

Patefield WM. **1981**. Algorithm AS 159: an efficient method of generating random R× C tables with given row and column totals. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* **30**: 91–97.

Pauw A. **2006**. Floral syndromes accurately predict pollination by a specialized oil-collecting bee (*Rediviva peringueyi*, Melittidae) in a guild of South African Orchids (Coryciinae). *American Journal of Botany* **93**: 917–926.

Pauw A, Stofberg J, Waterman RJ. **2009**. Flies and flowers in Darwin's race. *Evolution* **63**: 268–279.

Pauw A. **2013**. Can pollination niches facilitate plant coexistence? *Trends in Ecology and Evolution* **28**: 30–37.

Pellissier L, Alvarez N, Guisan A. **2012**. Pollinators as drivers of plant distribution and assemblage into communities. *Evolution of Plant–Pollinator Relationships*: 392–413.

Pelser PB, De Vos H, Theuring C, Beuerle T, Vrieling K, Hartmann T. **2005**. Frequent gain and loss of pyrrolizidine alkaloids in the evolution of Senecio section Jacobaea

(Asteraceae). *Phytochemistry* **66**: 1285–1295.

Petanidou T, Kallimanis AS, Tzanopoulos J, Sgardelis SP, Pantis JD. **2008**. Long-term observation of a pollination network: Fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology Letters* **11**: 564–575.

Peter CI, Johnson SD. **2014**. A pollinator shift explains floral divergence in an orchid species complex in South Africa. *Annals of botany* **113**: 277–288.

Phillips RD, Bohman B, Anthony JM, Krauss SL, Dixon KW, Peakall R. **2015**. Mismatch in the distribution of floral ecotypes and pollinators: Insights into the evolution of sexually deceptive orchids. *Journal of Evolutionary Biology* **28**: 601–612.

Podolsky RD. **1992**. Strange floral attractors - pollinator attraction and the evolution of plant sexual systems. *Science* **258**: 791–793.

Poisot T, Bever JD, Nemri A, Thrall PH, Hochberg ME. **2011**. A conceptual framework for the evolution of ecological specialisation. *Ecology Letters* **14**: 841–851.

Poole RW, Rathcke BJ. **1979**. Regularity, randomness, and aggregation in flowering phenologies. *Science* **203**: 470–471.

Prokop P, Fedor P. **2016**. Why do flowers close at night? Experiments with the Lesser celandine Ficaria verna Huds (Ranunculaceae). *Biological Journal of the Linnean Society* **118**: 698–702.

Pyke GH. **1982**. Local geographic distributions of bumble bees near Crested Butte, Colorado: competition and community structure. *Ecology* **63**: 555–573.

Pyke GH, Inouye DW, Thomson JD. **2012**. Local geographic distributions of bumble bees near Crested Butte, Colorado: competition and community structure revisited. *Environmental Entomology* **41**: 1332–1349.

R Core Team. **2016**. R: A language and environment for statistical computing.

Rabinowitz D, Rapp JK, Sork VL, *et al.* 1981. Phenological properties of wind- and insect-pollinated prairie plants. *Ecology* **62**: 49–56.

Raguso RA, Weiss MR. **2015**. Concerted changes in floral colour and scent, and the importance of spatio-temporal variation in floral volatiles. *Journal of the Indian Institute of Science* **95**: 69–92.

Rathcke B. **1983**. Competition and facilitation among plants for pollination In: Real L, ed. *Pollination biology*. Orlando: Academic Press Inc, 305–329.

Rees M, Condit R, Crawley M, Pacala S, Tilman D. **2001**. Long-term studies of vegetation dynamics. *Science* **293**: 650–655.

Reverté S, Retana J, Gómez JM, Bosch J. **2016**. Pollinators show flower colour preferences but flowers with similar colours do not attract similar pollinators. *Annals of Botany* **118**: 249–257.

Rivest SA, Austen EJ, Forrest JRK. **2017**. Foliage affects colour preference in bumblebees (*Bombus impatiens*): a test in a three-dimensional artificial environment. *Evolutionary Ecology* **31**: 435–446.

Rodríguez-Gironés MA., Santamaría L. **2004**. Why are so many bird flowers red? *PLoS Biology* **2**: 1515–1519.

Rodríguez I, Gumbert A, De Ibarra NH, Kunze J, Giurfa M. **2004**. Symmetry is in the eye of the "beeholder": Innate preference for bilateral symmetry in flower-naïve bumblebees. *Naturwissenschaften* **91**: 374–377.

Roth LSV, Balkenius A, Kelber A. **2008**. The absolute threshold of colour vision in the horse. *PLoS ONE* **3**: 1–6.

Runquist RB, Grossenbacher D, Porter S, Kay KM, Smith J. **2016**. Pollinator-mediated assemblage processes in California wildflowers. *Journal of Evolutionary Biology* **29**: 1045–1058.

Russell AL, Newman CR, Papaj DR. **2017**. White flowers finish last: pollen-foraging bumble bees show biased learning in a floral color polymorphism. *Evolutionary Ecology* **31**: 173–191.

Santamaría L, Rodríguez-Gironés MA. **2015**. Are flowers red in teeth and claw? Exploitation barriers and the antagonist nature of mutualisms. *Evolutionary Ecology* **29**: 311–322.

Sargent RD. **2004**. Floral symmetry affects speciation rates in angiosperms. *Proceedings of the Royal Society B: Biological Sciences* **271**: 603–608.

Sargent RD, Ackerly DD. **2008**. Plant-pollinator interactions and the assembly of plant communities. *Trends in Ecology and Evolution* **23**: 123–130.

Shrestha M, Dyer AG, Boyd-Gerny S, Wong BBM, Burd M. **2013**. Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *New Phytologist* **198**: 301–310.

Shrestha M, Dyer AG, Bhattarai P, Burd M. **2014**. Flower colour and phylogeny along an

altitudinal gradient in the Himalayas of Nepal. *Journal of Ecology* **102**: 126–135.

Shrestha M, Lunau K, Dorin A, *et al.* 2016. Floral colours in a world without birds and bees: the plants of Macquarie Island. *Plant Biology* **18**: 842–850.

Shuttleworth A, Johnson SD. **2009**. The importance of scent and nectar filters in a specialized wasp-pollination system. *Functional Ecology* **23**: 931–940.

Shuttleworth A, Johnson SD. **2010**. The missing stink: sulphur compounds can mediate a shift between fly and wasp pollination systems. *Proceedings of the Royal Society B: Biological Sciences* **277**: 2811–2819.

Siesser WG. 1980. Late Miocene origin ofthe Benguela upswelling system off northern Namibia. *Science* **208**: 283–285.

Smith SD, Rausher MD. 2011. Gene loss and parallel evolution contribute to species difference in flower color. *Molecular Biology and Evolution* **28(10)**: 2799-2810.

Snijman DA. **2013**. *Plants of the Greater Cape Floristic Region, Vol. 2: The Extra Cape Flora. Strelitzia 30*. Pretoria: South African National Biodiversity Institute.

Sõber V, Moora M, Teder T. **2010**. Florivores decrease pollinator visitation in a self-incompatible plant. *Basic and Applied Ecology* **11**: 669–675.

Spiesman BJ, Gratton C. **2016**. Flexible foraging shapes the topology of plant – pollinator interaction networks. *Ecology* **97**: 1431–1441.

Sprengel C K. 1793. *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen*. Рипол Классик.

Stebbins GL. **1970**. Adaptive radiation of reproductive characteristics in Angiosperms, I: pollination mechanisms. *Annual Review of Ecology and Systematics* **1**: 307–326.

Steiner KE, Whitehead VB. **1990**. Pollinator adaptation to oil-secreting flowers - *Rediviva* and *Diascia*. *Evolution* **44**: 1701–1707.

Stirton CH. **1983**. Nocturnal petal movements in the Asteraceae. *Bothalia* **14**: 1003–1006.

Strauss SY, Conner JK, Rush SL. **1996**. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *The American Naturalist* **147**: 1098–1107.

Strauss SY, Irwin RE, Lambrix VM. **2004**. Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. *Journal of Ecology* **92**: 132–141.

Strauss SY, Whittall JB. **2006**. Non-pollinator agents of selection on floral traits In: Harder LD, Barrett SC, eds. *Ecology and Evolution of Flowers*. Oxford: Oxford University Press, 120–139.

Strauss SY, Cacho NI. **2013**. Nowhere to Run, Nowhere to Hide: The Importance of Enemies and Apparency in Adaptation to Harsh Soil Environments. *The American Naturalist* **182**: E1–E14.

Strauss SY, Cacho NI, Schwartz MW, Schwartz AC, Burns KC. **2015**. Apparency revisited. *Entomologia Experimentalis et Applicata* **157**: 74–85.

Struck M. **1994**. Flowers and their insect visitors in the arid winter rainfall region of southern Africa: observations on permanent plots. Insect visitation behaviour. *Journal of Arid Environments* **28**: 51–74

Stuart-Fox D, Moussalli A, Whiting MJ. **2007**. Natural Selection on Social Signals: Signal Efficacy and the Evolution of Chameleon Display Coloration. *The American Naturalist* **170**: 916–930.

Sutherland JP, Sullivan MS, Poppy GM. **1999**. The influence of floral character on the. *Enthomologia Experimentalis et Applicata* **93**: 157–164.

Suzuki R, Shimodaira H. **2015**. pvclust: Hierarchical Clustering with P-values via multiscale Bootstrap Resampling.

Theis N, Lerdau M, Raguso RA. **2007**. The challenge of attracting pollinators while evading floral herbivores: patterns of fragrance emission in *Cirsium arvense* and *Cirsium repandum* (Asteraceae). *International Journal of Plant Sciences* **168**: 587–601.

Thompson JN. **1999**. Specific hypotheses on the geographic mosaic of coevolution. *The American Naturalist* **153**: S1–S14.

Tilman D. **1994**. Competition and biodiversity in spatially structured habitats. *Ecology* **75**: 2–16.

Tilman D. **2004**. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 10854–10861.

Troje N. **1993**. Spectral categories in the learning behaviour of blowflies. *Zeitschrift für Naturforschung* **48**: 96–104.

Tur C, Sáez A, Traveset A, Aizen MA. **2016**. Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south

Andean plant communities. *Ecology Letters*: 576–586.

Van Doorn WG, Van Meeteren U. **2003**. Flower opening and closure: a review. *Journal of Experimental Botany* **54**: 1801–1812.

Van Doorn WG, Kamdee C. **2014**. Flower opening and closure: an update. *Journal of Experimental Botany* **65**: 5749–5757.

Von Frisch K. 1965. Die tänze der bienen. In Tanzsprache und Orientierung der Bienen (pp. 3-330). Springer, Berlin, Heidelberg.

Von Hase A, Cowling RM, Ellis AG. **2006**. Petal movement in cape wildflowers protects pollen from exposure to moisture. *Plant Ecology* **184**: 75–87.

Vorobyev M, Osorio D. **1998**. Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society B: Biological Sciences* **265**: 351–358.

Wang H, Talavera M, Min Y, Flaven E, Imbert E. **2016**. Neutral processes contribute to patterns of spatial variation for flower colour in the Mediterranean *Iris lutescens* (Iridaceae). *Annals of Botany* **117**: 995–1007.

Warren J, Mackenzie S. **2001**. Why are all colour combinations not equally represented as flower-colour polymorphisms? *New Phytologist* **151**: 237–241.

Waser NM. **1978**. Interspecific pollen transfer and competition between co-occurring plant species. *Oecologia* **36**: 223–236.

Waser NM, Chittka L, Price M V, Williams NM, Ollerton J. **1996**. Generalization in pollination systems, and why it matters. *Ecology* **77**: 1043–1060.

Waterman RJ, Bidartondo MI, Stofberg J, Combs JK, Gebauer G, Savolainen V, Barraclough TG, Pauw A. 2011. The effects of above-and belowground mutualisms on orchid speciation and coexistence. *The American Naturalist* **177(2)**: E54-E68.

Wessinger CA, Rausher MD. **2012**. Lessons from flower colour evolution on targets of selection. *Journal of Experimental Botany* **63**: 5741–5749.

Wignall AE, Heiling AM, Cheng K, Herberstein ME. **2006**. Flower symmetry preferences in honeybees and their crab spider predators. *Ethology* **112**: 510–518.

Wolowski M, Carvalheiro G, Freitas L. **2017**. Influence of plant–pollinator interactions on the assembly of plant and hummingbird communities. *Journal of Ecology* **105**: 332–344.

Yeates DK, Greathead D. **1997**. The evolutionary pattern of host use in the Bombyliidae (Diptera): A diverse family of parasitoid flies. *Biological Journal of the Linnean Society* **60**: 149–185.

Zangerl AR, Berenbaum MR. **2009**. Effects of florivory on floral volatile emissions and pollination success in the wild parsnip. *Arthropod-Plant Interactions* **3**: 181–191

Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**: 686–693.

Zipf GK. **1949**. *Human behaviour and the principal of least effort*. Cambridge, Massachusetts: Addison-Wesley.

Zhao D, Tao J. 2015. Recent advances on the development and regulation of flower color in ornamental plants. *Frontiers in plant science* **6**:261.

Zufall RD, Rausher MD. 2004. Genetic changes associated with floral adaptation restrict future evolutionary potential. *Nature* **428(6985)**: 847.